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(3) cellular oxidation

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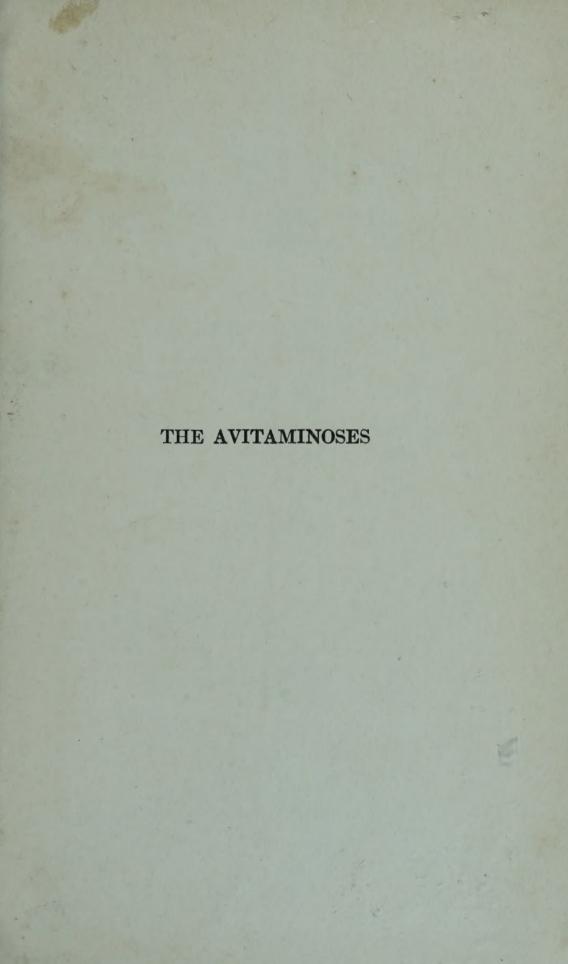
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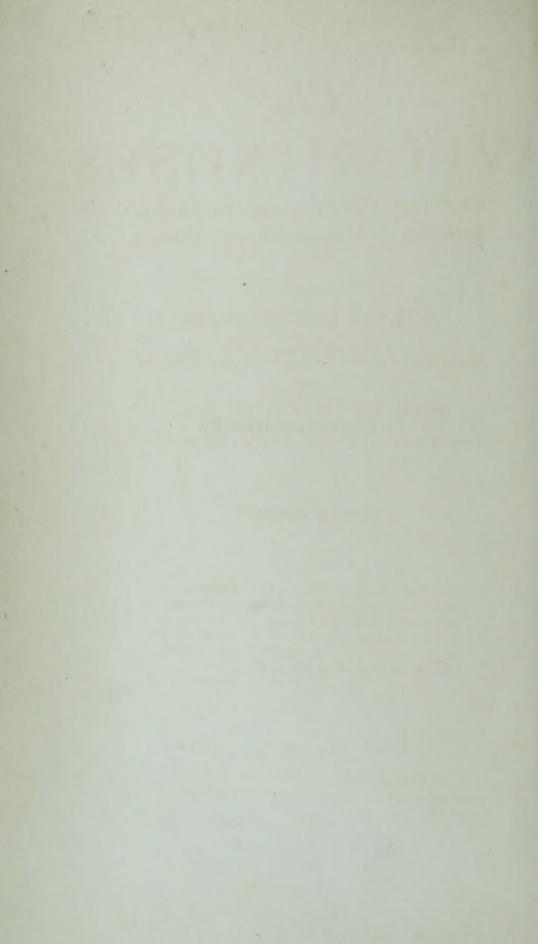
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# The (AVITAMINOSES)

The Chemical, Clinical and Pathological Aspects of the Vitamin Deficiency Diseases

By

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#### SECOND EDITION

"For it is now clear to anyone who will study the evidence, that nutrition has greater constructive potentiality than science had foreseen, and that even in the everyday choice of food we are dealing with values which are above price, for the health and efficiency, duration and dignity, of human life."

—HENRY C. SHERMAN

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### PREFACE TO THE SECOND EDITION

The present revision incorporates many advances in our understanding of the vitamins and has necessitated a complete re-writing of most of the text. There has been no change, however, in our general purpose of providing a small manual which would review the field from clinical, chemical and pathological points of view. This is of itself a considerable undertaking. Yet it seems a worthy one. Such a book should be useful not only to the tyro but also to those whose experience is strictly limited to the bio-chemical or clinical aspects of the problems of the deficiencies or to a single deficiency. Specialization to this degree is already common in the field.

The chief problem in writing such a book as this is in the selection of the material to be included. Our own choosing has been according to several rules. Isolated observations have been disregarded for the time being unless they appeared particularly important or useful to the discussion. In many cases we have been guided by unpublished information, in others by opinions and possibly prejudices we could not always defend. We have tried to maintain coördination and readability and this has often prevented us from citing reports which deviated from the theme under consideration. The endless references to authors which mark some reviews seem to serve no real purpose whatever and distract the reader.

However the alternative is not without fault also and as we go to press we are conscious of the many significant and excellent reports which have been omitted. In certain cases we may in this way contribute to a degree to the wastage of valuable information. The reading necessary to the preparation of this book has demonstrated to us how serious this is. Many observations are constantly being made which are mere

repetitions of work well done years ago. The tremendous volume of investigations in the field makes us profligate and countless careful studies remain neglected simply because our

attention cannot encompass them all.

We have not hesitated to speculate along certain lines but these lapses are obvious and the critical reader may skip them. We have been eager to identify underlying principles and mechanisms and to define their application and limitations. This is very true of the lesions of the deficiency diseases. How specific are they? Which may be considered pathognomonic of a particular deficiency wherever we find it? Certain subjects have been included simply because they seemed to contribute towards a solution of this problem.

The chapter on cellular oxidation has been written for those readers whose academic training antedates the advances which have been made in the chemistry of oxidation. To the initiated this will be useless. The appendix has been limited, as in the first edition, to methods which we have used. certain instances this experience has been restricted but in every case the technique has been examined both from the point of view of theoretical and practical application and studies have been made with the method on specimens from patients and the sources of difficulties have been carefully considered. The tables of vitamin values of foods have been completely revised according to the best sources with which we are familiar. Such tables are in a strict sense highly inaccurate. The values vary with the variety and breed, season, soil conditions, methods of picking and preparing or storing and canning. Moreover the assay methods on which they are based are themselves open to criticism. These limitations, which many disregard, cannot be avoided. Despite them such tables have a usefulness which makes their inclusion desirable.

A considerable number of new illustrations have been added and the references have been checked for accuracy. We will appreciate criticism of all phases of the book and especially solicit corrections of errors which may have crept in at some stage in the preparation of the manuscript.

To those who have contributed material, have read manuscript and proof or aided in other ways we extend our sincere thanks.

W. H. E. G. D.



### PREFACE TO FIRST EDITION

This book is directly derived from "The Vitamine Manual" which the senior author wrote fifteen years ago. But the scope of the book has been enlarged by the addition of both pathological and clinical discussions of the vitamin deficiency diseases. How this came about may be of interest for it mirrors the transitional development of the subject. Eight years ago the senior author brought to the pathological laboratory of the old New York Hospital some guinea pigs he suspected of being scorbutic although there was nothing in their gross appearance to support such a diagnosis. In this way did the advantages of joint pathological and biochemical studies become apparent to us and the association begin which has led us to write this book.

The problems in which we have since collaborated have dealt particularly with the development of anatomical criteria by which to evaluate the results of dietary experiments and, more recently, the application of this knowledge to the practice of medicine including the search for anatomical evidence of deficiency disease in human tissues at autopsy and in the wards and clinics.

The book has been planned to be a helpful manual rather than a complete treatise. In addition to the current views concerning the nature and functions of the vitamins we have tried to assemble in one volume what is known of the pathological anatomy and clinical aspects of the diseases due to their insufficiency, to describe clinical methods we have found useful in studying human cases and to present tables of vitamin values of all those foods which have been assayed expressed not in plus signs but in units per ounce so that diets can be more precisely measured for their vitamin value.

While we have not hesitated to interject our own views of controversial subjects we believe we have identified them as being our own clearly enough that the reader may subtract them from the rest of the book and still retain a resume of current opinion of other workers.

Our hope is that the book may be as useful to others as the many workers in this field have been to us in helpful advice and other generous services. The most pleasurable experience which has come to us in the writing of the following pages has been due to the universal friendliness and helpful interest of our colleagues. It is a rich and undeserved reward. We thank them all at this time.

WALTER H. EDDY, Ph.D. GILBERT DALLDORF, M.D.

### FOREWORD

The preparation of the first comprehensive treatise in English on the pathological responses to vitamin deficiencies emphasizes the importance of the application of morphological control in the study of vitamins. It emphasizes the commanding position of morphology in the interpretation of disease The occurrence of a great variety of degenerative diseases of organs and mucous membranes, of peculiar character and obscure etiology, was long recognized as one of the mysteries of pathology. Rickets, scurvy, beriberi and pellagra were but the more prominent of these diseases. Their general features were fully recognized but their causation remained undetermined until the introduction of the doctrine of vita-Then began, on a vast scale, the experimental study of vitamins, vielding a volume of new facts of great scope, but lacking coordination and uniformity. This situation was mainly due to the lack of any delicate and reliable indicator for vitamin deficiency.

Commencing with the work of Wolbach and Howe in 1925–1928, demonstrating the specific changes in the morphology of various epithelial tissues due to vitamin A deficiency, the value of pathological examination as a delicate indicator of the presence or absence of adequate vitamins was demonstrated and extensive studies were stimulated. It is in this field that the authors of the present treatise have made important contributions and the present volume demonstrates how these methods have served to bring order out of chaos.

The enormous literature on vitamins has been collated and digested and reliable conclusions presented in condensed and accessible form for the general physician and pathologist. It will now be possible to determine more accurately how fre-

quently deficiency diseases occur among the sick in general and hospital practice. A reliable clue is also available to the general pathologist in the interpretation of various morbid conditions, metaplasia, disorders of collagen formation, proliferative changes of non-inflammatory type, degenerative changes in the nervous system, etc.

The book is comprehensive in its inclusion of a discussion of the nature of the various vitamins and their functions as well as other information of a very practical nature. The appearance of such a work, therefore, is to be cordially welcomed, and the authors congratulated on its preparation.

JAMES EWING.

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## PART I THE VITAMINS AND THE AVITAMINOSES



### CHAPTER I

### VITAMINS AND DISEASE

There is nothing new in the conception that inadequate diets cause disease. Even primitive peoples recognize this relationship and provide special foods to prevent and cure certain diseases. Price's experience would indeed suggest that in some respects the wisdom of primitive man is superior to our knowledge for it insures better dental development and physique and provides more effectively for the nutritional requirements of pregnancy and puberty. But the dietary prescriptions of natives are strictly empirical, rich in both fallacy and truth.

In our early history are many illuminating comments on deficiency diseases. In calling our dogs Toby we keep alive the story of that blind Tobiah who was guided by his dog until an Archangel prescribed fish liver for his xerophthalmia and caused the skin to fall from his eyes. Early physicians knew something of these diseases. Beriberi was described in the 7th century, scurvy in the 13th and pellagra in the 18th. The curative powers of certain foods became evident many years ago. Lind's successes in treating scurvy are two hundred years old and the beneficial effect of cod liver oil in rickets was generally recognized twenty years before vitamin D was discovered. Perhaps the most prophetic observation of all is that of Budd, who, nearly a hundred years ago wrote that scurvy was due to the "lack of an essential element which it is hardly too sanguine to state will be discovered by organic chemistry or the experiments of physiologists in a not too distant future."

Many clues to the entire field of the avitaminoses may be

found in the literature of the years that followed. These were translated into facts as a direct result of the studies of Liebig and Lavoisier which made inevitable an investigation of the qualitative aspects of nutrition. A clear conception of the subject and its importance and the groundwork for its methods of study were laid by two outstanding chemists, Funk and Hopkins. Funk, in 1911, extracted from rice polishings a few milligrams of a crystalline substance which was curative of experimental beriberi and coined the name "vitamine." He also predicted with remarkable accuracy which diseases would prove to be due to "vitamine" deficiency. Hopkins, in the years from 1906 to 1912 established the groundwork of our technique and clearly foresaw the importance of the accessory food substances and the future course of developments.

Funk compounded the word "vitamine" of the prefix "vita" and the suffix "amine" because his original crystals contained basic or amine nitrogen and were essential to life. Moreover, "it was necessary for me to choose a name that would sound well and serve as a catchword." The term was modified by dropping the final "e" at the suggestion of Drummond when it was learned that an amine group was not a characteristic of "vitamines." The common practice of designating the various vitamins by letters is a sequel of the pioneer nutritional studies which started at the University of Wisconsin in 1907 as an investigation of the value of cereals in animal nutrition and which were later transferred to Johns Hopkins University. There, in 1913, McCollum and Davis demonstrated a growth factor in the non-saponifiable fraction of the fat from butter and egg yolk which they called "unidentified dietary factor, fat-soluble A."

At present three methods are used in designating the vitamins. Thus the substance which Funk originally called "vitamine" is known as water soluble vitamin B<sub>1</sub>, the anti-beriberi or anti-neuritic vitamin or as thiamin in this country and aneurin

on the continent. The latter terms are the chemical names for the substance and it is probable that as the various vitamins are identified chemically their chemical names will replace the terms by which we first knew them.

Table 1 gives the vitamins that have been postulated and for which evidence of existence is well established. At the time of printing, we have included in this list only those that appear to have a definite effect on animals. There have also been discovered a number of substances which exercise similar functions in the control of the growth of plants or lower organisms. It may be that ultimately some of these will be shown to have a direct effect on human beings. An example of this is the recent discovery that the biotin which stimulates the growth of certain fungi, the co-enzyme R which was shown to have an effect on the roots of legumes and the anti-egg white injury factor designated as vitamin H by Parsons and György, appear to be identical.

At the present time, however, there is general agreement to designate plant hormones by special names rather than include them in the list of vitamins, at least until the time they are demonstrated to play a rôle in human or animal nutrition.

As shown in the following chapter there is little structural resemblance between the individual vitamins, vitamin A having quite a different chemical configuration than that of vitamin B<sub>1</sub> or vitamin D. Vitamin is a functional designation not a chemically descriptive term. It is useful, however, to consider them together for they share certain features. Thus they are present, and active, in minute amounts, in contrast to the considerable quantities of the ordinary nutrients. They also differ from the latter in that they may be inactivated by heat and oxidation. They have been discovered and studied by a common technique, that of devising basal diets of purified constituents deficient only in the factors under investigation and so measuring the effects of deprivation and of supplements. Because they resemble in their behavior

TABLE 1
Postulated Vitamins

|  | Postulated Vitamit  |   |
|--|---|---|
| ALPHABETICAL DESIGNATION   | CHEMICAL NAME   | PHYSIOLOGICAL<br>CHARACTERIZATION   |
| True A   | Activated carotene Activated carotene Thiamin or aneurin  | Antixerophthalmic factor<br>Antixerophthalmic factor<br>Antineuritic or Anti-beri-<br>beri factor |
| Vitamin B <sub>2</sub> or G<br>Vitamin B <sub>4</sub>                | Riboflavin<br>Williams-Waterman<br>factor                 | Anticheilosis factor Bird weight maintenance factor   |
| Vitamin B <sub>4</sub>   | Reader factor   | Rat paralysis preventive factor   |
| Vitamin B <sub>5</sub>   | Peters' factor  | Heat stable, pigeon<br>weight maintenance<br>factor   |
| Vitamin B <sub>6</sub> or filtrate<br>factor I<br>Filtrate factor II | Adermin or pyridoxin<br>Pantothenic acid                  | Rat anti-acrodynia factor Juke's anti-bird derma- titis factor                                    |
| Vitamin C  | Ascorbic acid Calciferol Activated 7 dehydro- cholesterol | Antiscorbutic factor Antirachitic factor Antirachitic factor                                      |
| Vitamin E<br>Vitamin H   | Tocopherol Biotin, coenzyme R                             | Antisterility factor Anti-egg white-dermatitis factor   |
| Vitamin J<br>Vitamin K   |   | Antipneumonia factor<br>Blood coagulation factor  |
| Vitamins L <sub>1</sub> and L <sub>2</sub>                           |   | Japanese lactation fac-   |
| Vitamin M  | Day's factor  | Anti-monkey pellagra  |
| Vitamin P  | Citrin or eriodictyol                                     | factor Anti-hemorrhagic diathesis factor  |
| Vitamin U<br>Vitamin W<br>Vitamin P-P                                |   | Chick growth factor Rat growth factor Antipellagra or black tongue factor                         |

In addition to the above there are certain terms that have been used and discarded, certain terms that have been duplicated, and certain terms that have been postulated pending further substantiation.

the products of the glands of internal secretion, such as thyroxin and insulin and are present in foods they are sometimes called food hormones. Of recent years the importance of certain vitamins to plant growth and development has suggested that they may represent the equivalent, in the vegetable kingdom, of the hormones of the animal kingdom and thus be both plant hormones as well as food hormones.

A further characteristic of certain vitamins is that they occur in natural sources in a physiologically inactive form (provitamins) and become active only after conversion within the animal. Thus vitamin A exists in plants as a yellow pigment, carotene, which is activated in the liver. The D vitamins are the result of ultra violet light activation of parent substances.

The avitaminoses are as ill assorted a group of diseases as the vitamins are ill assorted chemically. But they, too, share enough common characteristics to justify their inclusion in a single group of diseases. They are due to the absence of minute amounts of biologically important materials rather than to the presence of minute amounts of infectious agents. They do not cause disease in a positive but in a negative sense. genic organisms produce chemical and morphological damage; vitamin deficiency removes an essential ingredient from the physiological equation. The deficiency is the disease. true that many deficiency diseases are recognized by the compensatory mechanisms of the body. Thus in riboflavin deficiency the cornea becomes vascularized because, it is thought, the natural method of oxygen exchange in that part is suppressed. But these are secondary manifestations of the disease.

A third characteristic of the deficiency diseases is that they may be present in any degree. One may suffer a latent infection but not be partially infected. A malignant growth is present or not. But the deficiency diseases may occur in such partial forms as to raise the question whether disease is

actually present. This is, indeed, the most pressing problem in the field for all evaluation of what constitutes a proper diet rests upon it. The avitaminoses are commonly present in

TABLE 2
Some Vitamin Units and Reference Standards

| VITAMINS           | BIOLOGICAL UNIT  | REFERENCE STANDARDS   |
|--------------------|--|---|
| A                  | The amount of source capable of producing the effect of 0.0006 mgm. pure beta carotene   | U.S.P. Reference Cod Liver<br>Oil containing 3000 In-<br>ternational units of A<br>per gram |
| $\mathrm{B}_{1}$   | The amount of source capable of producing the effect of 0.003 mgm. pure crystalline thiamin  | Crystalline thiamin   |
| B <sub>2</sub> (G) | The amount of source capable of producing the effect of 0.0025 mgm. pure riboflavin (this is known as the Bourquin-Sherman unit, no International unit having as yet been defined) | Pure riboflavin   |
| $\mathbf{B}_{6}$   | Amount of source necessary to cure<br>acrodynia in 3 weeks (Schneider,<br>Ascham, Platz, Steenbock unit)   | Probably 0.1 mgm. pure crystalline B <sub>6</sub>   |
| C                  | The amount of source capable of producing the effect of 0.05 mgm. pure l-ascorbic acid   | Pure l-ascorbic acid  |
| D                  | The amount of source capable of producing the antirachitic effect of 0.000025 mgm. pure calciferol   | U.S. Ref. Oil containing 95<br>units D per gram   |
| E                  | The Evans unit; amount of source necessary in 21 days of rat gestation to ensure birth of litte:   | Standardized wheat germ<br>oil  |
| K                  | Dam unit is the amount of source<br>necessary per gram of the animal on<br>three successive days in order to<br>bring chick blood clotting time to<br>normal                       | Alfalfa meal extract,<br>standardized or syn-<br>thetic 3 methyl, 1,4 naph-<br>thoquinone   |
| P-P                | Expressed in milligrams of nicotinic acid  | Pure nicotinic acid   |

mild degree, a feature which more than any other delayed recognition of their importance. In these incipient forms the lesions and symptoms are difficult to recognize, the effects of treatment less obvious and uncertainties bound to remain. They exist as a disturbance of the substrate, as a coloration of the physiological and structural constitution which, while of great importance in balancing the scales of health and in modifying the responses to noxious agents, remains hidden from view.

### TABLE 3 Vitamin Unit Equivalents

Owing to different usages in the past, the following table (3) of equivalents may also be of value in translating unitage into International unit equivalents for vitamins A, B<sub>1</sub>, C, and D.

VITAMIN A

1 Int. unit = 0.0006 mgm. Beta-carotene

1 Int. unit = 0.7 Sherman-Munsell units

VITAMIN B<sub>1</sub>

1 Int. unit = 0.003 mgm. thiamin

1 Int. unit = 2 Sherman-Chase units

1 Int. unit = 0.5 Smith curative units

1 Int. unit = 1.0 Chick-Roscoe units

1 Int. unit = 20.0 Cowgill mgm.-equivalents

VITAMIN C

1 Int. unit = 0.05 mgm. ascorbic acid

1 Int. unit = 0.1 Sherman-LaMer unit

VITAMIN D

1 Int. unit = 0.000025 mgm. calciferol

1 Int. unit = 1 U.S.P. unit

1 Int. unit = 0.37 Steenbock units

1 Int. unit = 1 A.D.M.A. unit

1 Int. unit = 1 Oslo or Poulsson unit

N.B.: Viosterol is frequently labelled 250 or 150 D. This means containing 250 to 150 times the content of cod liver oil. All Viosterol preparations must contain at least 10,000 Int. units of D per gram.

A further characteristic is that they are seldom the immediate cause of death. One searches the mortality records in vain for clues to their importance and in the dissecting room they receive scant attention. They are contributory causes of death but seldom the immediate cause. Despite the little attention they receive in necropsy protocols those deficiency diseases which are well understood from a morphological

point of view have characteristics as unique and specific as the structure of the vitamins themselves.

It is generally believed that the important vitamins have now been discovered. Wormall wrote: "The vitamin story may now be regarded as approaching the end of what is possibly the most important chapter, that dealing with the identification of those vitamins which are required for the prevention of certain widespread deficiency diseases in man. This statement does not mean that all the vitamins required by man have been discovered...but...it will be rather surprising if some serious vitamin deficiency disease has so far been missed." Recent developments suggest however that certain manifestations of deficiency diseases are not yet recognized and a continued lively development of the clinical study of the deficiency states is very likely.

It has likewise become obvious that the deficiency diseases are a much more complex problem for the physician than for the biochemist since the manifestations of deficiency may be influenced by other diseases just as other diseases modify vitamin requirements, by altering the efficiency of assimilation and suppressing or emphasizing the importance of dietary deficiencies. There is, indeed, a special field of nutrition which is strictly limited to the practice of medicine.

The major importance of this new science will be found in the practice of preventive medicine and in the field of public health. That this is now well recognized is shown by the extensive investigations and outstanding discoveries of members of the United States Public Health Service. National institutes of nutrition have been established in certain countries and the League of Nations devoted much effort to the subject. The goal of all these efforts will be the improvement of physical constitution, resistance to disease and a healthier and happier people. It should be a great reward for that army of chemists and physicians who have explored the subject and charted our course.

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### CHAPTER II

### THE CHEMICAL NATURE OF THE VITAMINS

The following list of vitamins gives those that have been chemically identified and given names descriptive of their composition or effect. In several instances the chemical identification has been confirmed by synthesis.

The chemical nature of the above vitamins is described in the following pages. In general, it is of interest to note that they are quite unrelated chemically and that many have now been shown to form essential parts of cellular enzyme systems concerned in the processes of cellular oxidations and reductions, the so-called respiratory enzyme systems.

### THE CHEMICAL NATURE OF VITAMIN A

Clue to the nature of this vitamin came with the demonstration that it occurs in plant sources as a yellow pigment; first identified by Wachenroder in 1826 and called by him carotene because he obtained the pigment from carrots. Steenbock in this country suggested that there might be a relation between carotene and vitamin A but proof that carotene is provitamin A is due primarily to the work of Moore and of Karrer and associates. At that time it was well known that there are a series of yellow carotenoid pigments in plants, especially in the chlorophyll-rich parts of plants. Not all of these, however, proved capable of conversion into vitamins A. Palmer gives the accompanying table (table 5) of these compounds and their relation to vitamin A.

As shown by Palmer's table, studies of these pigments narrowed the list of provitamins A down to four, viz. the three carotenes (alpha, beta, and gamma) and cryptoxanthin. Of

these, beta-carotene yielded the maximum vitamin A effect per unit of weight.

TABLE 4
Some Chemically Identified Vitamins

Vitamins A<sub>1</sub> and A<sub>2</sub>: Activated carotene Vitamin B<sub>1</sub>: Thiamine or aneurin Vitamin B<sub>2</sub> or G: Riboflavin Vitamin B<sub>6</sub>: Adermin or pyridoxine Filtrate Factor II: Pantothenic acid

Vitamin P-P: Nicotinic acid Vitamin C: Ascorbic acid Vitamin D: Activated sterols

Vitamins E: Alpha- and beta-tocopherol Vitamins K: Substituted 1,4 naphthoquinones

Vitamin P: Citrin or eriodictyol

TABLE 5
Carotenoids, Source and Vitamin A Potency

| NAMES           | WHERE FOUND                            | MOLECULES OF<br>VITAMIN A<br>PRODUCIBLE<br>FROM ONE<br>MOLECULE OF<br>PIGMENT |
|-----------------|--|---|
| Beta-carotene   | Green leaves, carrots, butter          | Two   |
| Alpha-carotene  | Carrots, chestnut leaves, red palm oil | One   |
| Gamma-carotene. | Lily of the valley leaves              | One   |
| Cryptoxanthin   | Yellow corn, egg yolk, green grass     | One   |
| Zraxanthin      | Yellow corn, egg yolk, green leaves    | None  |
| Rhodoxanthin    | Seed coats of the yew                  | None  |
| Capoxanthin     | Red peppers, pimiento                  | None  |
| Fucoxanthin     | Brown algae                            | ?   |
| Taraxanthin     | Sunflower, dandelion                   | None  |
| Violaxanthin    | Yellow pansy                           | None  |
| Flavoxanthin    | Buttercup                              | None  |
| Xanthophyll     | Green leaves and grass                 | None  |
| Lycopene        | Red tomato, watermelon                 | None  |
| Astacin         | Lobster, salmon, shrimp                | None  |

The carotenes are strongly yellow compounds. It was already known from study of fish liver oils that active vitamin

A is nearly colorless. What happens when the provitamin beta-carotene is changed to active vitamins A?

The explanation was worked out in several laboratories. As stated above, Steenbock in 1919 had shown that yellow corn was a more potent source of vitamin A than white corn and suggested that vitamin A might be a leuco form

Note that this formula, if broken in two at point A, would give two identically constructed molecules. In the liver this is accomplished by hydrolytic cleavage at point A, i.e., breaking by addition of water. The result, 2 molecules of vitamin A from one molecule of beta-carotene having the following formula:

### (b) Vitamin A formula:

FIG. 1. WHAT HAPPENS WHEN BETA-CAROTENE BECOMES VITAMIN A

of carotene. Von Euler in Stockholm fed carotene to rats and demonstrated its vitamin A activity and his work was confirmed by Dr. Thomas Moore in Cambridge, England, who proved definitely that the activity was produced by changing the carotene into the nearly colorless vitamin A.

To Karrer and his associates we owe the determination of the chemical constitution of plant carotene and fish oil A and what happens when one is changed into the other. Figure 1 shows this process in terms of chemical structure.

We have called attention in figure 1 to the symmetry of beta-carotene and the production of identical halves by cleavage at the middle point, thus yielding two molecules of the vitamin A.¹ Why do alpha and gamma carotene and crypto-xanthin produce only one molecule of vitamin A per molecule of provitamin? Compare the formulae of the compounds shown in figure 2 with that of beta-carotene and the reason becomes clear. It is evident that unlike beta-carotene the ring compounds at the right of point A are in every case different from the ring at the left of A and it is the left ring that must be present to give us the vitamin A molecule. Hence these compounds supply only one molecule of A per molecule of carotenoid pigment. The ring in the vitamin A molecule is known as optically inactive beta-ionone.

Quite recently it was discovered that the vitamin A obtained from the livers of fresh water fish, while giving the same physiologic effect as that from salt water fish, differed from it in certain chemical and physical reactions. Study revealed that the fresh water fish vitamin A had in it two more carbon atoms than the salt water fish vitamin A. The two forms are therefore now designated as vitamin A<sub>1</sub> (salt-water fish vitamin A) and A<sub>2</sub> (fresh-water fish vitamin A). Their comparative structure is shown in figure 3.

That the optically inactive beta-ionone ring plays a part in the ability of vitamin A to produce physiologic effect is demonstrated by the difference in behavior shown by beta and alpha carotene as just explained.

There is also evidence that the double bonds in the straight chain (polyene chain) part are involved in conferring physiologic activity. If we hydrogenate cod liver oil it loses its potency. Hydrogenation doesn't affect the ring compound

<sup>&</sup>lt;sup>1</sup> The actual demonstration of the formation of 2 molecules of vitamin A from 1 of carotene has not yet been made.

Optically Inactive Beta-Ionone (beta-ionone ring) structure but does eliminate the double bonds in the polyene chain. Oxidation has a similar effect and vitamin A activity is destroyed by oxidation.

Just why these structural changes vary the physiologic response still remains to be explained.

There is, however, one more point to be noted before leaving this discussion of vitamin A chemistry. If you will refer once more to figure 1, you will note that when beta-carotene

(a) Vitamin 
$$A_1$$
 $CH_3$ 
 $CH_3$ 
 $CH_3$ 
 $CH_3$ 
 $CH_3$ 
 $CH_3$ 
 $CH_4$ 
 $H_2C$ 
 $CC_-C=C-C=C-C=C-C+2OH$ 
 $CC_-CH_4$ 
 $CC_-CH_4$ 

FIG. 3. Two Forms of Active VITAMIN A

is broken in two by addition of water to form two molecules of vitamin A the vitamin retains part of the water to form the alcohol group (—CH<sub>2</sub>OH). Alcohols form esters when the OH group is replaced by another organic group. Some of the variation in fish oils as sources of vitamin A is today attributed to their containing vitamin A in ester form instead of in the free alcohol form. These esters in general appear to have more physiologic potency than the vitamin in the free alcohol form.

Vitamin A and carotenoids have the ability to absorb certain wave lengths of light. This characteristic makes it possible to determine their presence in a source and to estimate quantitatively the amount present by suitable spectrographic instruments. Vitamin  $A_1$  has a specific absorption for wave length 328 m $\mu$  and  $A_2$  for wave length 345 m $\mu$ .

Both carotene and the vitamins A react with antimony trichloride to produce a color with a specific absorption band, e.g.:

> Beta-carotene at 590 m $\mu$ Vitamin A<sub>1</sub> at 620 m $\mu$ Vitamin A<sub>2</sub> at 623 m $\mu$

This color reaction permits colorimetric determination of vitamin and carotene content and is now being employed for assay of the vitamin A and carotene content of biological fluids such as blood and urine.

Beta-carotene is a slightly bitter tasting, reddish-yellow substance, soluble in fats and fat solvents. Vitamin A is a pale yellow, viscous oil, also fat soluble. Both are destroyed as to physiologic activity by oxidation and must be protected from such oxidation to retain stability and physiological potency.

# THE CHEMICAL NATURE OF VITAMIN B1, THIAMIN

Funk's crystalline "vitamine" of 1911 undoubtedly contained vitamin B<sub>1</sub> but it was not pure B<sub>1</sub>. Jansen and Donath first successfully isolated the pure vitamin B<sub>1</sub>. By a modification of Jansen and Donath's procedure, R. R. Williams and associates first succeeded in increasing yields of the vitamin from rice polishings and ultimately determined its structural chemistry. Williams and Cline succeeded further in synthesizing the vitamin and the synthetic compound is now abundantly available. The synthesis was almost simultaneously accomplished in three other laboratories (Andersag and Westphal at Elberfeld, Germany, 1937; Todd and Bergel at Edinburgh, 1937; and Hoshimo and Ohta in Japan, 1937).

The Council of Pharmacy of the American Medical Association has adopted William's suggested name, Thiamin. On the continent the product is called by the name suggested by Jansen, Aneurin.

Figure 4 shows the structural formula deduced by R. R. Williams and its relation to two other compounds which have proved of importance in explaining its physiological action

# (a) Thiamin chloride

# (b) Co-carboxylase (thiamin pyrophosphate)

#### (c) Thiochrome

$$\begin{array}{c} N = C - N = C \\ C = CH_2 - CH_2 - CH_2OH \\ CH_3 = C \\ N = CH \end{array}$$

FIG. 4. THIAMIN AND RELATED PRODUCTS

and in assay for vitamin B<sub>1</sub> potency; co-carboxylase and thiochrome.

Examination of figure 4 justifies Funk for assigning the suffix "amine" to the anti-neuritic vitamin since it contains the amine (NH<sub>2</sub>) group. Basically it is a union of two compounds, a pyrimidine base attached to a nitrogen-carbon-sulfur ring containing a pentavalent nitrogen. This compound's nature was suggested by H. T. Clarke as probably a thiazole.

On treatment with sulfite thiamin separates into two nuclei and this phenomenon made its chemical identification possible. The reaction which can also be accomplished with nitrite is as follows:

$$I \\ C_{12}H_{18}N_4SOCl_2 + Na_2SO_3 \rightarrow C_6H_9N_3SO_3 + C_6H_9NSO + 2NaCl$$

Fraction I was proven to contain the pyrimidine nucleus.

Fraction II was shown to contain the thiazole nucleus by the collaboration of Clarke and Gurin.

Some years ago, Morgan showed that sulfuring of fruits reduced their vitamin B<sub>1</sub> content. The explanation is now clear. The sulfur fumes uniting with water formed sulfite which actually split the vitamin in two and thus inactivated it.

In 1937 Löhman and Schuster isolated a crystalline substance from yeast which in combination with an enzyme system containing the protein of washed yeast cells, de-carboxylated pyruvic acid, thus:

$$CH_8$$
— $CO$ — $COOH$  ——>  $CH_8$ — $CHO$  +  $CO_2$ 

Löhman and Schuster named their crystalline product cocarboxylase.

Meanwhile Peters and associates at Oxford (see Chapter VII) had already suggested that thiamin functions in an enzyme system concerned with carbohydrate metabolism and with pyruvic acid breakdown in particular. Demonstration that Löhman's co-carboxylase was phosphorylated thiamin went far toward completion of the explanation of the rôle of thiamin in carbohydrate metabolism.

In natural sources and in biological fluids vitamin B<sub>1</sub> may exist as the free base or in the co-carboxylase condition necessitating assay for both forms to establish vitamin B<sub>1</sub> potency. Melnick and associates state that the thiamin in urine is all in the non-phosphorylated form.

When either thiamin or co-carboxylase are oxidized by alkaline ferricyanide a substance is formed having a strong blue fluorescence. This substance is thiochrome. With a fluorimeter and suitable standards for comparison it is possible to assay the amount of thiochrome in a solution. This fact makes it possible to assay vitamin B<sub>1</sub> sources by first converting the vitamin present to thiochrome and constitutes an assay procedure being used quite extensively today.

Thiamin also reacts with the reagent called diazotized paraaminoacetphenone to form a colored dye whose amount can be determined colorimetrically. This chemical method of estimating the vitamin B<sub>1</sub> content of urines has been used by Melnick as cited above, to determine when the body is saturated with this vitamin.

The biological test for B<sub>1</sub> is still required by the U.S. Department of Agriculture, Food and Drug Administration, to establish B<sub>1</sub> content of drug and food products and description of the rat method required will be found in the U.S. Pharmacopeia XI, 1939 supplement.

Thiamin chloride in pure form is a white crystalline substance having a slightly bitter, salty taste. It is readily soluble in water and its physiological activity is destructible by heat, especially in alkaline solution. Elvehjem reports the following percentage destruction in the cooking of meats:

| Beef by broiling        | 50% destruction  |
|-------------------------|------------------|
| Beef by roasting        | 61% destruction  |
| veal by frying          | 450% destruction |
| veal by roasting        | 5807 dostruction |
| Pork loin by frying     | 2507 doctmention |
| Pork loin by roasting.  | 50% destruction  |
| Beef kidney by stewing. | 4007 destruction |
|                         | 40% destruction  |

The U.S.P. or International unit of vitamin B<sub>1</sub> is the equivalent of 0.00333 mgm. of crystalline thiamin.

# THE CHEMICAL NATURE OF VITAMIN B2 OR G

Booher obtained water-soluble, pigmented substances from whey powder that when added to a basal diet devised by Bourquin and Sherman for use in estimating vitamin G potency, proved growth stimulating. The presence of this vitamin in skim milk was therefore early realized. In the same year, Kuhn and coworkers isolated from dried egg albumen a water-soluble, green-fluorescent pigment which, like Booher's concentrates, stimulated the growth of rats on a Bourquin-Sherman basal diet in amounts as low as 0.1 mgm. per day. This substance was not new to chemists for it had been isolated and described by Blyth in 1879 and further described by Bleyer and Kallman in 1925.

Fig. 5. The Structure of Vitamin B2 or G (Riboflavin)

Kuhn and coworkers carried the study of it still further, suggested its flavin structure and also its possible relation to the "yellow ferment" of Warburg and Christian. They recovered it from liver as well as from milk and egg and these compounds were named hepato-, lacto-, ovo-flavins respectively.

Later, however, these pigments from the different sources were found to have similar structure; a sugar called d-ribose attached to a tricyclic chromophore nucleus. As a result the use of the terms, lacto, hepato, etc., have been dropped and the substance, regardless of source, is called ribose-flavin or riboflavin. Its chemical structure is shown in figure 5.

In 1932, Warburg and Christian reported the extraction of a respiratory ferment from yeast. From its color it was called the yellow ferment. Theorell showed this enzyme to have the structural configuration shown in figure 6.

If you compare figures 5 and 6, it becomes evident that riboflavin is a structural element in Warburg's yellow ferment. As that ferment is widely distributed in tissue cells and is essential to oxidation-reduction reactions in the metabolism of these cells, the importance of a continuous supply of riboflavin for normal cell activity becomes apparent.

$$\begin{array}{c} CH_2 \\ CHOH \\ CHOH \\ CHOH \\ CH_2 \\ H \\ C \\ N \\ N \\ \end{array} \begin{array}{c} PO(OH)_2 \\ \\ CH_3 - C \\ C \\ CH_4 - C \\ C \\ CH_5 - C \\ C \\ CH \\ \end{array} \begin{array}{c} Protein \\ \\ CO \\ \end{array}$$

Fig. 6. Theorell's Configuration of Warburg's Yellow Ferment

Pure riboflavin is an orange-yellow crystalline substance with a bitter taste. It is soluble in water and its activity is resistant to heat destruction. Strong alkalis destroy its activity and it is also sensitive to light, being irreversibly decomposed by ultra-violet irradiation. It is not destroyed by oxidation.

There is evidence (Kuhn) that physiological activity depends on the character of the side chain and the presence of at least one methyl group on the carbon ring. There is also definite evidence that riboflavin must be phosphorylated before it can function physiologically (Laszt and Verzar). The phosphorylated riboflavin is called cytoflav.

Riboflavin has a characteristic yellow-green fluorescence in neutral solutions and this property has been utilized to devise a fluorimetric assay method (Supplee). The bioassay method of Sherman and Bourquin is still standard for potency using, today, pure riboflavin as the reference standard. It is now a requirement of the U. S. Food and Drug Administration that potency in label declarations must be in micrograms (gamma) of riboflavin per unit of weight of product. Older preparations expressing potencies in Sherman-Bourquin units may be converted into such declaration by assuming the S-Bunit as equivalent to 2.5 micrograms of pure riboflavin.

## THE CHEMICAL NATURE OF THE PELLAGRA-PREVENTIVE VITAMIN

Early work on the differentiation of water-soluble vitamin B indicated a pellagra-preventing as well as a beriberi preventive vitamin in the complex (Goldberger et al.). The demonstration of the riboflavin as distinct from the anti-neuritic thiamin first inclined to the belief that riboflavin was the anti-pellagric substance. Further study disproved this and revealed the water-soluble B complex of McCollum and Kennedy to contain several vitamins and that one of these was specific against human pellagra. That this substance was nicotinic acid or its amide was first established by Elvehjem and coworkers.

Nicotinic acid as a chemical entity was, of course, well known long before its identification with pellagra-prevention. It is a pyridine carboxylic acid with the structure shown in figure 7.

The acid is a moderately sour, white crystalline substance, soluble in water and heat resistant.

Like riboflavin, nicotinic acid has been shown (Euler, Warburg) to be an essential constituent of enzyme systems that cells use in oxidation and reduction. Vilter utilized the fact that H. influenzae requires as a growth factor a substance containing nicotinic acid in the prosthetic group to devise a test for this substance in human blood. His test showed

marked reduction in the factor in pellagrin blood when contrasted with that of normal individuals and prompt restoration to normal content in pellagrin blood with nicotinic acid treatment.

However, it should be made clear that while nicotinic acid and some of its compounds (amide, sodium nicotinate, coramine) heal pellagrous glossitis, stomatitis, vaginitis, urethritis, and protect and restore the porphyrin content of the urine to normal there are other symptoms frequently manifested by pellagrins such as polyneuritis and cheilosis that require other members of the B-complex (e.g., thiamin and riboflavin).

FIG. 7. NICOTINIC ACID AND AMIDE

In substantiating claims of anti-pellagric potency labels must state the potency in milligrams of nicotinic acid; there is no U.S.P. or International pellagra-preventive vitamin unit. At present, comparisons are largely made by tests on dogs with blacktongue and determination of amounts of natural products necessary for their cure.

Elvehjem has reviewed the relation of nicotinic acid to pellagra and the attempts at chemical assay of nicotinic acid content by various methods. He states that to date "the method that appears to be most satisfactory depends upon the breakdown of the pyridine nucleus with cyanogen bromide and aniline to give a yellow colored compound which can be measured colorimetrically (Pearson), (Kringstad and Naess)."

# THE CHEMICAL NATURE OF VITAMIN B6

A specific type of rat skin lesion attended by a pink or florid dermatitis (acrodynia) permitted the differentiation of another vitamin from the water-soluble B complex. This was first described as Filtrate factor I (Lepkovsky, Jukes and Krause), and is apparently identical with the "H" of Booher, the "H" of Hogan and Richardson, and the "Y" factor of Chick and Copping but not with the "H" of György and Parsons.

The vitamin was isolated and chemically identified in six laboratories in the same year (Lepkovsky, György, Kuhn, Ichiba, Emerson, Keresztesy). Keresztesy and Stevens in the Merck Laboratory reported the empirical formula of the isolated vitamin as C<sub>8</sub>H<sub>11</sub>NO<sub>2</sub> and Harris and Folkers confirmed this by synthesis and established the structure shown

Fig. 8. VITAMIN B6 (ADERMIN OR PYRIDOXIN)

in figure 8. This compound, like the isolated vitamin, cured acrodynia in rats in 14 days with a dosage of 0.1 mgm.

The product crystallized out as white platelets. As will be seen from figure 8, we have in this as in nicotinic acid, the pyridine ring with certain side chains. The crystals are salty tasting, water-soluble, and relatively resistant to heat. The product is stable to strong acids, to alkali and to nitrous acid. Kuhn found it non-dialyzable from yeast and this may indicate its combination with a protein in the natural source and its probable association with an oxidation enzyme system.

There is as yet no official definition of a unit of B<sub>6</sub> or a satisfactory chemical assay method. Biological assays have been devised and used in at least three laboratories (György, Wilson and Roy, Quackenbush et al., and Schneider et al). Schneider et al. define the unit as the amount of source necessary to cure acrodynia of moderate severity in a rat in

3 weeks. They have reported extensive assays of common foodstuffs expressing potency in this unit. György and Wilson and Roy used a slightly different unit, viz. amount necessary to cure acrodynia in 2 weeks. They found that amount to be 0.1 mgm. of pure pyridoxin. An interesting feature of their distribution report is the high potency of certain oils in this factor, wheat germ oil, for example, containing 250 units per gram as against 0.4 units per gram in milk.

That this vitamin is of value in human physiology has been strikingly demonstrated by Spies in treating certain symptoms in pellagrins not responsive to nicotinic acid, thiamin or riboflavin treatment. The vitamin may be associated with fatty acid metabolism. Birch could find no evidence of combination of the vitamin with lipids but rather that there is a functional relation between the vitamin and the unsaturated fatty acids.

### CHEMICAL NATURE OF FILTRATE FACTOR II

If, in fractionating the water-soluble B complex, one removes first thiamin and riboflavin and then eliminates all other adsorbent materials there is left a filtrate that still shows vitamin activity. From this filtrate fraction, B<sub>6</sub> (pyridoxin or adermin) was first separated. The fraction remaining was still preventive of a type of dermatitis developed by chicks and for this chick dermatitis-preventive factor, Lepkovsky and Jukes suggested the tentative name of filtrate factor II.

R. J. Williams and coworkers have described a compound which they call pantothenic acid because of its universal distribution in plants and animals and its essentiality for growth of all living cells. Williams reported its isolation as the calcium salt to which he assigned the empirical formula  $(C_8H_{14}NO_5)_2Ca$ .

Its structural formula was determined by coöperative effort of chemists of the Merck Laboratories and R. J. Williams

(Science 91, 246, 1940). It is formed by the reaction of  $\alpha$ -hydroxy- $\beta$ ,  $\beta$ -dimethyl- $\gamma$ -butyrolactone and  $\beta$ -alanine and has the following structural formula:

$$\begin{array}{c|c} CH_{\$} & OH \\ & | & | \\ HOCH_2--C---CH--CO--NHCH_2--CH_2--COOH \\ & | \\ CH_{\$} \end{array}$$

Interest in this acid is increased by the report by Jukes and Wooley that filtrate factor II is identical with Williams' pantothenic acid or at least contains this compound.

TABLE 6
Chemical Status B Complex—Components, 1940

| CHEMICALLY IDENTIFIED   | STILL UNIDENTIFIED  |  |
|---|---|--|
| B <sub>1</sub> or Thiamine or Aneurin B <sub>2</sub> or Riboflavin B <sub>6</sub> or Adermin or Pyridoxine P-P or Nicotinic Acid Filtrate Factor II or Pantothenic Acid | B <sub>3</sub> or Gizzard erosion factor B <sub>4</sub> —Anti-rat paralysis factor B <sub>5</sub> —Peters, bird maintenance factor W or Elvehjem rat growth factor U—Chick growth factor Anti-gray hair factor Anti-spectacled eye factor |  |

György and Eckhardt have stated that in the presence of B<sub>1</sub>, B<sub>6</sub>, and riboflavin three further types of dermatologic conditions not corrected by these factors have been observed. They therefore suggested that the term "adermin" for B<sub>6</sub> was badly chosen and suggested the term "pyridoxin" as appropriate.

In reviewing the chemistry of the water-soluble B complex we may therefore separate the compounds at present chemically identified as shown in table 6.

# THE CHEMICAL NATURE OF VITAMIN C

The elucidation of the chemical nature of the antiscorbutic vitamin is a product of studies in many laboratories. In

1932, King and Waugh obtained from lemon juice an actively antiscorbutic substance apparently identical chemically with the "hexuronic acid" recovered from adrenal cortex, oranges and cabbage by Szent-Györgyi. The identity of hexuronic acid as the antiscorbutic vitamin itself was announced by Svirbely and Szent-Györgyi and by King and coworkers in 1932–33. Its structure was established by Haworth, Hirst and collaborators and in the same year, Reichstein, Grussner and Oppenhauer synthesized it.

Szent-Györgyi's isolation of vitamin C was a consequence of his study of oxidation systems. In his lectures he gives the following account:

The more I learned about this new substance, the more interesting it seemed to be. Eventually, I crystallized it, that is to say, peeled it out in a pure condition which made analysis possible. It was an acid and it seemed to be related to an unknown sugar which I called "Ignose," the substance itself being called "Ignosic Acid." But the editor of the journal to whom I sent my paper did not like jokes and rejected the name. "Godnose" being no more successful we agreed that the child's name should be "hexuronic acid." Later with advancing knowledge of its structure it had to be rebaptized in haste and it is now called ascorbic acid (sometimes, cevitamic acid) because it is identical with vitamin C and prevents scurvy. In this way I became a father without wishing it, the father of a vitamin. Such accidents seem to happen even in science.

The outstanding characteristic of ascorbic acid is that when oxidized the reaction proceeds in two steps; the first step being reversible, the second step irreversible. The structure of l-ascorbic acid and these oxidative changes are shown in figure 9.

It was early shown that the most characteristic feature of vitamin C was the rather rapid destruction of physiological activity by oxidation, especially when heated in an alkaline or neutral solution. We know now that this is due to the chemical changes shown in figure 9. In natural sources the active vitamin occurs mainly in the fully reduced form (l-ascorbic acid). If oxidation is not too severe, the l-ascorbic acid may simply lose 2 hydrogens and go over to the dehydro-

ascorbic acid form. This form is convertible back to l-ascorbic acid by reducing agents such as H<sub>2</sub>S and can also be accomplished in the body, hence ascorbic acid eaten in either the reduced or dehydro-form is available for human use as an anti-scorbutic.

If, however, the oxidation proceeds farther than the dehydro stage no reversion to l-ascorbic acid is possible and physiologic activity is lost.

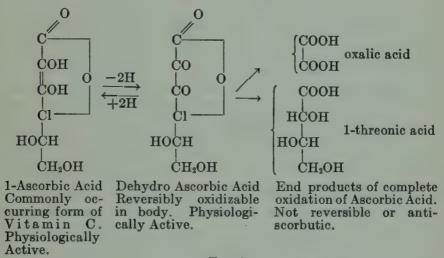


Fig. 9

In assay of vitamin C sources, therefore, it is necessary to determine their content of both l-ascorbic and dehydro-ascorbic acids.

l-Ascorbic and dehydro-ascorbic acids are not, however, the only compounds with antiscorbutic properties though they appear to be the principal forms of the vitamin in foods and biological materials. The following forms have been synthesized and tested for antiscorbutic value with the results noted (Zilva):

l-rhamno-ascorbic acid; one-fifth the potency of l-ascorbic acid.
l-arabo-ascorbic acid; one-twentieth the potency of l-ascorbic acid.
d-ascorbic acid; no potency.
d-gluco ascorbic acid; no potency.
d-galacto ascorbic acid; no potency.

It would appear from these products and their behavior that one essential to antiscorbutic activity of these sugar acids is associated with the position of the oxygen ring, the d-forms being inactive, the l-forms active. This ring position is not the only factor concerned with physiological activity, however, for while the l-rhamno and l-arabo forms have the ring in the proper position they exhibit only one-fifth to one-twentieth the potency of l-ascorbic acid itself.

The response of l-ascorbic acid to an oxidation-reduction indicator was first utilized by Tillmans and Hirsch to distinguish true fruit juices from artificial juices. They did not know at the time to what the response was due but using phenol-indophenol as an indicator they got strong reactions with fresh fruit juice, less reaction with stale juices, and no reaction with synthetic imitations of fruit juices. In the years 1930-33, Tillmans and associates, Zilva and others used the phenol-indophenol indicator to demonstrate presence of vitamin C activity and it was finally shown that by suitable precautions to exclude other reducing substances, such as glutathione and cysteine, the test could be made specific for quantitative assay of vitamin C content of both foodstuffs and biological fluids such as urine, blood, feces, and spinal fluid. Bessey gives the steps of the reaction with the indicator, 2,6, dichloro-phenol-indophenol (see figure 10):

Crystals of vitamin C (l-ascorbic acid) are now available by synthesis. In the dry state they are stable to air and light and retain their full antiscorbutic activity over long periods. In water solution aerobic oxidation takes place; accelerated by heat, light, presence of riboflavin and of certain ions; notably copper, but including iron, mercury, silver, manganese, ferricyanide, iodine, phosphotungstate, nitrate and peroxide. A specific oxidase destructive of vitamin C has been described in certain plant juices. King states, however, that this is a copper-protein complex and the copper is the destructive

factor.

In the food supply of this vitamin, interest centers in retention of stability under different methods of marketing and serving and unless certain precautions and controls are exercised oxidative destruction may severely reduce vitamin C potency between harvesting and serving of such sources.

Fig. 10. Reaction of Ascorbic Acid with Indicator Dye (After Bessy, J. A. M. A. 111: 1290, 1938)

There is also danger of loss by leaching when foods are cooked in water. Maintenance of low temperatures and avoidance of alkalies such as soda in vegetable cooking reduces oxidative destruction.

From the chemical viewpoint, the manipulation of either food or therapeutic sources of vitamin C must take steps to

effectively prevent or to reduce oxidation to the minimum compatible with retention of the product's natural properties.

In the chemical assay of vitamin C potency by use of the indophenol indicator the principal problem is the elimination of the other reducing substances. Evelyn has developed methods to this end and Farmer and Abt have developed micro methods applicable to blood and urine.

Vitamin C being now available for reference in bioassays or titrations, the U.S.P. or International unit has been put at 0.05 mgm. pure l-ascorbic acid. It is approximately one-

tenth of the Sherman unit formerly used in bioassays.

# THE CHEMICAL NATURE OF VITAMIN P

Szent-Györgyi and his coworkers in Hungary found that while in certain pathological cases the tendency of capillaries to bleed (hemorrhagic diathesis) could be reduced by treatment with l-ascorbic acid; certain natural vegetable juices, notably paprika juice, showed superiority over the use of the synthetic vitamin C alone. They sought for another hemorrhage controlling factor and believed they had found it in a flavonol fraction to which they gave the name of vitamin P.

Of this discovery and choice of name Szent-Györgyi writes as follows:

In citrus fruits we found an especially active member of this group (flavonols) present as a glucoside which up to that time had been unknown in this form. We called it with V. Bruckner, "Eriodictin."... In the unripe plant we find this substance in a methylated, inactive and stable form, which was known as "Hesperidin" for a long time;

# and again:

I had a letter from an Austrian colleague who was suffering from a severe hemorrhagic diathesis (vascular type). He wanted to try ascorbic acid in his condition. Possessing at that time no sufficient quantities of crystalline ascorbic acid, I sent him a preparation of paprika that contained much ascorbic acid and the man was cured by it. Later, with my friend, St.

Rusznyak, we tried to produce the same therapeutic effect in similar conditions with pure ascorbic acid but we obtained no response. It was evident that the action of paprika was due to some other substance present in this plant. It would have been a hopeless job to try and find and isolate this substance had we not had our experience with flavons. So we set out to prepare flavons, in the first place eriodictin, that can be easily injected and we found that similar pathological conditions, not previously amenable to therapy, could be cured by it with regularity. The effect had several characteristics of vitamin-action, so, tentatively, I called it "Vitamin P" in honor of Paprika and Permeability, on which later it was found to have an influence. As yet, I have failed to demonstrate its vitamin nature by animal experiments and until such proof is given the vitamin nature of this substance is not beyond doubt.

FIG. 11. STRUCTURE OF ERIODICTYOL (VITAMIN P)

The active fraction from lemon juice containing this factor has been called citrine. Lorenz and Arnold have prepared what they call a medicinal lemonade containing the citrine fraction in amounts suitable for therapeutic oral administration. They state that the water extract of whole lemon contains 1.7 mgm. citrine flavonols per gram of lemon. Lorenz emphasizes that the citrine fraction is a complex containing several compounds, not all of which are active.

The chemical structure assigned to "eriodictin" or "erio-

dictyol" is shown in figure 11.

Better identification of citrine and comparisons of flavonols for physiologic effects are necessary to establish these compounds in the vitamin group. The existence of a vitamin P having physiologic activity is controversial.

# THE CHEMICAL NATURE OF THE VITAMINS D

The search for the substance corrective of rickets has led to date to postulation (Bills) of at least ten compounds of a sterol type that have rickets-healing potency. With one exception all of these substances require ultraviolet irradiation to convert them from the physiologically inactive provitamin to the cure effecting form.

All these compounds contain a nucleus to which the chemist applies the name sterol. The nature of this sterol nucleus and the change produced in it by activation is shown in figure 12.

Fig. 12. The Sterol Nucleus of Vitamins D Before and After Activation

As shown in figure 12, the presence of the (HO) group makes the sterol a type of alcohol and activation appears to involve opening of the ring between positions 9 and 10.

The study of sterols has assumed added importance in recent years from the discovery that not only are they present in biological substances such as gall stones (cholesterol) and in vitamins D but also form the nucleus of certain of the sex hormones and certain carcinogenic substances. The bile acids also contain sterol groups as do certain cardiac poisons such as strophanthin and bufotoxins.

Tanret isolated a substance from ergot to which he gave the name ergot-sterol or ergosterol. It was this compound that was proven by the work of Hess, Steenbock, Rosenheim and Webster, Windaus and Hess, to be the substance in certain products which became antirachitic on ultraviolet irradiation and for some time it was supposed to be the only provitamin D. The isolation of active ergosterol from its irradiation products in 1931 and 1932 by Askew et al. and by Windaus made possible the understanding of the chemical nature of the provitamin and the vitamin. What this chemical relationship is, is shown graphically in figure 13.

For some time it was believed that there was only one vitamin D, viz. activated ergosterol or calciferol. Thanks to physiological behavior of chicks in contrast to rats as noted by Waddell, it became evident that the form of vitamin D in cod liver oil was not (mainly at least) of the ergosterol type. This led to the discovery of the form we know today as 7-dehydro-cholesterol. The character of this vitamin and its relation to ordinary cholesterol is shown in figure 14.

7-dehydro-cholesterol and activated ergosterol or calciferol

constitute the most common forms of vitamin D known today. The former appears to predominate in fish liver oils and in the human skin. The latter is found in materials such as yeast and ergot and is the form present in milk of cows fed irradiated yeast. The activated ergosterol is also the form purchased under the name of "Viosterol."

Fig. 14. Relation of Cholesterol to Vitamin D<sub>3</sub> (7-dehydro-cholesterol)

The reader will probably be curious as to what  $D_1$  is, since activated ergosterol is called  $D_2$  and activated 7-dehydrocholesterol is called  $D_3$ . There is no vitamin  $D_1$  in the literature today, the compound called by that name by German workers having proved to be a mixture of calciferol and lumisterol.

When the provitamin ergosterol is irradiated not all of it is converted into calciferol, ordinarily not more than 50 per

cent under ideal conditions. During the process of irradiation a series of compounds are formed which in order of appearance are listed by Bills as follows:

- 1. Ergosterol
- 2. Lumisterol
- 3. Tachysterol
- 4. Calciferol
- 5. Toxisterol (Substance 248)
- 6. Suprasterols I and II

From such irradiation mixtures lumisterol, the two suprasterols and calciferol have been isolated in crystalline state. Tachysterol has been separated as a benzoate. Toxisterol has not been isolated in pure state. It gets the name "Substance 248" because of an absorption band at 248 mu. Of all these compounds only calciferol has antirachitic action. Lumisterol is converted to calciferol and also may form with it an addition compound consisting of 1 part lumisterol to 1 part of calciferol. It was this addition product German workers originally classed as vitamin D<sub>1</sub>. Toxisterol is not antirachitic and may produce toxic effect; similarly tachysterol is non-antirachitic and may be slightly toxic. The suprasterols are non-antirachitic but only slightly toxic. Earlier preparations of irradiated ergosterol sometimes produced toxic effect now believed to be due to failure to eliminate the toxisterols and tachysterols.

As stated above, vitamins D<sub>2</sub> and D<sub>3</sub> appear to be the most abundant forms of the antirachitic factor. Bills, however, postulates at least 8 others. His list is shown in table 7.

What happens when the provitamins D are activated by ultraviolet light is still not entirely clear. Milas and Anderson state that they have evidence indicating that the physiological activity produced by irradiation involves not only a break in the ring between positions 9 and 10 and the double bonds in the ring but is also affected by the character of the side chain.

Knudson and associates have successfully activated ergos-

terol by bombardment with cathode rays. Moore and De Vries accomplished activation with radium emanations. Neither method is as effective as use of ultraviolet light.

X-rays and short length radio waves of high intensity are without effect. The greatest region of absorption appears to be between 305 and 230 m $\mu$  and these wave lengths have the greatest activating power though activation is possible up to 313 m $\mu$ .

# TABLE 7 Antirachitic Sterols (After Bills)

A. Structural formulae elucidated

Calciferol-Vitamin D2-activated ergosterol

7-dehydro-cholesterol—Vitamin  $D_3$ 

22-dihydro-ergosterol—Vitamin  $D_4$ 

7-dehydro-sitosterol—Vitamin D<sub>5</sub>

Cholesterilene sulfonate-Vitamin D6

B. Structural formulae not elucidated

Irradiated 7-hydroxy cholesterol

Cholesterol freed of normal provitamin and irradiated but not heated

Ergosterol heated with nitrites

Irradiated, heated reaction product of 7-ketocholesteryl acetate and isobutyl magnesium bromide

Irradiated 22, 23, oxido-ergosterol.

N.B.: D4 may be the significant antirachitic in irradiated cereals.

The conditions under which ergosterol is activated influence results. Solvents are a factor, ether solutions giving higher potency than alcohol or cyclohexane solutions. Activation is more effective on solutions than when applied to the dry material.

The U. S. Pharmacopeia provides a rat test and a unit in terms of rat response for label potency of vitamin D preparations. They also provide a reference standard cod liver oil established to contain per gram 95 units of antirachitic vitamin D. This agrees with the International unit of vitamin D which is 0.000025 mgm. pure Calciferol.

Since the rat test is used for labelling purpose and calciferol and 7-dehydro-cholesterol appear to produce identical healing in the rat, the unit of  $D_2$  and  $D_3$  may be stated as 0.000025 mgm. of irradiated crystalline  $D_2$  and  $D_3$ . Since for chicks  $D_3$  is more potent than  $D_2$ , the A.O.A.C. chick unit is defined as the antirachitic value for chicks of  $\frac{1}{8^{15}}$  gram reference cod liver oil.

# THE CHEMICAL NATURE OF VITAMIN E

The antisterility vitamin first called "X" by Evans and Bishop and later changed to "E" at the suggestion of Sure, is a chemical substance or group of substances whose nature was suggested by proof of its anti-oxidant potency (Olcott and Mattill).

By use of cyanic acid it is possible to make combinations of this acid with certain alcohols. These compounds are called allophanates. By boiling an allophanate with dilute methyl alcoholic potassium hydroxide the alcohol can be split off from the allophanate and its identification made possible.

In 1936, Evans and the Emersons succeeded in separating from a wheat germ oil concentrate three allophanates. Two of these proved biologically active; one was inactive. The alcohol separated from one of these biologically active allophanates had the empirical formula  $(C_{29}H_{50}O_2)$  and to it they gave the name alpha-tocopherol. The other, less active compound they called beta-tocopherol. Subsequent study indicated it to have one less  $CH_2$  group than the alpha type and an empirical formula of  $C_{28}H_{48}O_2$ .

On the basis of the antioxidant power of these compounds Fernholz suggested that alpha-tocopherol might be a monether of durohydroquinone (see fig. 15). This was later abandoned and in 1938, Fernholz suggested that alpha-tocopherol had the structure shown in figure 15. His suggestion was confirmed by John in the same year. Bergel and associates suggest for beta-tocopherol a slightly different nucleus leaving the nature of the side chain to be developed.

a-Tocopherol acc. to Fernholz (1938); Chromane nucleus

FIG. 15. FORM OF VITAMIN E

In potency 1.2 mgm. alpha-tocopherol is equivalent to 1.9 mgm. beta-tocopherol according to Bacharach.

Several other compounds have now been synthesized with vitamin E activity and Karrer and Jensen claim that the biologic activity is determined in part by the character of the side chain and in part by the CH<sub>3</sub> groups in the ring. Its antioxidant potency is probably associated with the OH group in the ring.

It will be noted that the formula given in figure 15 shows the OH group, concurring with the evidence of Olcott and Drum-

TABLE 8

Comparison of Potentiometric Estimation of Vitamin E and Acetyl Values

| NAME OF OIL | ACETYL NUMBERS | TOCOPHEROL IN NON-<br>SAP. PORTION, ACCORD-<br>ING TO KARRER |
|-------------|----------------|--|
|             |                | per cent   |
| Wheat germ  | 14.4           | 13.4   |
| Maize germ  | 11.0-11.5      | 10.2   |
| Olive oil   | 10.6           | 0.9  |
| Linseed oil | 4.0            | 2.34   |
| Palm oil    | 8.4-1.9        | 0.55   |

In this series only olive oils fail to show a proportionate relation to tocopherol.

mond that vitamin E possesses an OH group. That this group accounts for antioxidant but not for biologic activity of the vitamin is further confirmed by the fact that acetylation destroys antioxidant effect but does not affect biologic potency, for acetylation acts on the OH group.

Vitamin E potency is usually expressed in "Evans" units and determined biologically. The Evans unit is the amount of E or E source just necessary during the gestation period of 21 days to insure the production of a litter by a previously non-productive female rat. Thus by the Evans system a 525 mgm. oil is one of which 525 mgm. must be fed during the

21 day gestation period to produce a litter in a female rat

previously proven unable to produce such a litter.

Mattill (1938) suggested that since the acetyl number of an oil indicates its OH groups, and since vitamin E contains OH groups, the acetyl number might be an index of potential vitamin E content (see table 8).

Emmerie and Engel making use of the knowledge that vitamin E is destroyed by such an oxidant as ferric chloride with reduction of the iron and the added fact that the alpha-dipyridil reagent measures only non-reduced iron have devised a colorimetric test for vitamin E using these reagents. They claim to eliminate substances other than E that might reduce iron by use of a preliminary treatment with a special adsorbent called Floridin XS Earth.

Furter and Meyer claim to produce an oxonium salt with intense red color, estimable photometrically by treatment of alpha-tocopherol with concentrated HNO<sub>3</sub> in absolute alcohol with short heating.

Karrer et al. reported it possible to estimate tocopherol potency by potentiometric titration with gold chloride—2 mols AuCl₃ equivalent to 3 mols of alpha-tocopherol. In table 8, we give some values obtained by Karrer with his potentiometric method checked against the acetyl numbers of the same oils.

Vitamin E is stable at high temperature, up to 250°C. in dry condition, according to Evans. Aeration at 97°C. does not destroy it but rancid fats are destructive, hence aeration at high temperatures may first induce fat rancidity and indirectly bring about the destruction of E.

Vitamin E is stable to ordinary light but destroyed by prolonged ultraviolet irradiation.

Hydrogenation does not destroy activity but bromination does. Cooking or drying by steam distillation does not have harmful effect but certain vigorous oxidants cause destruction.

No toxic effects have been observed with large doses of the vitamin.

#### THE CHEMICAL NATURE OF VITAMIN K

This vitamin controls the production of blood prothrombin and hence one of the factors necessary to the clotting of blood.

Blood clotting in general is assumed to proceed by two steps:

Step 1. Prothrombin + thromboplastin + ionized calcium forms the enzyme thrombin.

The calcium is supplied by food; the thromboplastin or cephalin by the blood platelets and tissues; the prothrombin probably by the liver.

Step 2. Fibrinogen + thrombin forms fibrin.

# (a) Natural vitamin K1; 2 methyl, 3 phytyl 1,4 Naphthoquinone

# (b) 1,4 Naphthoquinone

Fig. 16. Forms of Natural Vitamin K and their Relation to 1,4
Naphthoquinone

In 1929–30, Dam noted that chicks on certain diets developed hemorrhages, had abnormally long clotting time and low prothrombin content of the blood. He found that these conditions could be corrected by feeding dried spinach or alfalfa and in 1935 postulated the existence of a fat-soluble vitamin which he called the "Koagulation Vitamin" or vitamin K.

The elucidation of the chemical structure of vitamin K has been amazingly rapid. The significant nucleus of these vitamins is the compound known as naphthoquinone (see fig. 16).

K<sub>1</sub> as obtained from alfalfa is a light yellow oil crystallizing from acetone or alcohol at low temperatures. It has absorp-

tion maxima at 243, 248, 261, 270, 323 mµ.

 $K_2$  obtained from putrid sardine meal is a light yellow crystalline substance with a melting point of 50.5–2. It has absorption maxima at 249, 261, 269, 430 m $\mu$ .

The vitamins are found in the non-sterol fraction of the non-saponifiable fraction.

Fieser has confirmed the structure of natural K<sub>1</sub> by synthesis.

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# CHAPTER III

# VITAMINS AND CELLULAR OXIDATION

The existence of vitamins was first demonstrated by the appearance of certain specific symptoms such as beriberi, scurvy, night blindness, etc., and the proof that these conditions are curable or preventable by minute amounts of some dietary factor, hence the names antiscorbutic, anti-beriberi vitamin as shown in table 1 in Chapter I.

With advancing knowledge of the chemical nature and structure of the vitamin molecules it has been shown that many of these appear to take part in the oxidation-reduction processes which constitute the metabolism of tissue cells. This latter discovery tends to justify the term that has been sometimes applied to these substances, namely food hormones, for in their relations to food metabolism they behave much like the principles evolved by the endocrine glands and there is some evidence that the vitamins may even be concerned in the production of hormones.

Their relation to oxidation and reduction also indicates that they may be directly concerned in the maintenance of normal behavior of tissue cells and that in many cases the specific symptoms resulting from a prolonged inadequacy of these factors may be merely the result of disturbance of metabolism in one or more tissues; that in some cases the symptoms appear in regions quite separate from the tissues where the vitamin lack starts the disturbance. We know, for example, that in beriberi or polyneuritis there is an increase of pyruvic acid in the blood stream due not to lack of vitamin B<sub>1</sub> in the blood but to failure in tissue cells of sufficient vitamin B<sub>1</sub> to convert the pyruvic acid to water and carbon dioxide or again; a lack



of vitamin D in the bone tissue may result in the throwing into the blood of an abnormal amount of the phosphate ester

splitting enzyme, phosphatase.

To have a clear picture of the relation of vitamins to cellular oxidation requires a discussion of the character of this process as it is revealed by present studies of this reaction. Therefore, before proceeding to the discussion of the relation of vitamin deficiency to specific symptoms, there is outlined in the following pages a discussion of this matter of cellular oxidation and reduction.

### THE PROCESS OF OXIDATION

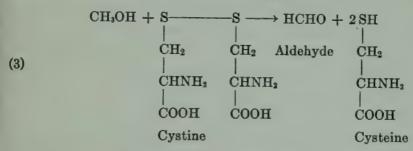
It is common knowledge that certain substances spontaneously react with the oxygen of the air to form oxides. Coal burns to form carbon monoxide or carbon dioxide. Metals corrode to form metallic oxides, but foodstuffs, as we know them, are generally indifferent to the action of the oxygen of the air or as the chemist calls it, to molecular oxygen. When therefore we write equation (1) to show what happens to sugar in the body, this equation fails to express anything except the end result and no such reaction takes place between the substance sugar by simple exposure to the molecular oxygen:

(1) 
$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + calories$$
 
$$Sugar + oxygen \longrightarrow oxides + energy$$

We also know today that oxidation does not always mean a chemical union between oxygen and the substance oxidized. We call, for example, the conversion of alcohol to aldehyde, a process of oxidation but what the oxygen actually does to the alcohol is to remove from it 2 hydrogen atoms:

(2) 
$$\begin{array}{c} \text{CH}_{\bullet}\text{OH} + \text{O} \longrightarrow \text{HCHO} + \text{H}_{\bullet}\text{O} \\ & \text{Alcohol} \stackrel{-2\text{H}}{\longrightarrow} \text{Aldehyde} \end{array}$$

In such an operation we say that the oxygen has acted as a hydrogen acceptor and it follows that any substance that can act as a hydrogen acceptor can do the same thing that oxygen itself has done and hence accomplishes an oxidative process. Certain sulfur compounds found in living cells may and do so function, for example, amino acids known as cystine and glutathione (see equation (3)):



Furthermore the cysteine formed in this reaction can again be brought back to the cystine form (see equation (4)) by in turn giving up its hydrogen to oxygen.

With the aid of cystine then, we could picture continuous conversion of alcohol to aldehyde as taking place in the following stages: First, the alcohol losing its hydrogen to the cystine thus converting that substance into cysteine; second, the oxygen itself taking the hydrogen from the cysteine and converting it back to cystine which can then remove 2 more hydrogens from the alcohol. In that case the cystine acts as a carrier of hydrogen between the alcohol and the oxygen.

But there is still another process to which we give the name of oxidation today. An atom consists of a central nucleus containing a positively charged particle or particles called protons, surrounded by a collection of negatively charged particles called electrons. The simplest atom we know today

is the hydrogen atom which appears to consist of a single positively charged proton and a single negatively charged electron. It is obvious that if such an atom were to lose its negative particle, the electron, it would be left with a positive charge. It has been found possible by various means to remove from atoms or to add to atoms one or more electrons. The resulting substances are called ions and ions may have excess of negative or positive charge. The hydrogen ion for example would have one positive charge. The iron atom, by losing 2 electrons, has two positive charges and by losing a third electron would carry three positive charges. This variation in the amount and kind of charge of these ions is the present basis of valence in chemistry. In other words, valence may be defined as the number of electrons that an atom of that element loses or takes up in entering into combination with atoms of other elements.

Since hydrogen has only one electron to lose, the hydrogen ion has a valence of one or one positive charge. Oxygen atom can acquire 2 electrons under certain conditions and therefore carries 2 negative charges. In the formation of water, therefore, 2 univalent positively charged hydrogen ions can unite with one divalent negatively charged oxygen ion to form a molecule of water. By our first definition of oxidation, the reaction of oxygen with hydrogen to form water is an oxidation of the hydrogen. If then we have a substance which can accept electrons from atoms of hydrogen, or for that matter from any other atoms, we might generalize and call it an oxidizing agent. Likewise, we would, in the most general sense, say that loss of electrons on the part of any atom constitutes oxidation. This may be called a definition of oxidation in terms of electrovalence.

It is true that in the formation of molecules of many substances, even in the case of water, the electrons are not completely transferred from one atom to another but are shared between them. This type of transfer, resulting in what is

called *covalence*, is, nevertheless, similar to the electrovalence described above, and an atom that has lost a share in one or more of its electrons, due to their partial transfer to another atom, may be looked upon as having become oxidized. The recipient atom, acquiring a share in electrons not formerly its own, may be said to have been reduced.

With these three definitions of oxidation in mind, let us see what actually happens inside a tissue cell when it receives a fuel source of energy such as sugar.

#### ACTIVATION

It was Warburg who first showed that something had to happen to molecular or atmospheric oxygen before it could be made to take up either hydrogen or electrons. When the substrate was sugar, he found that the activation of molecular oxygen to make it receptive could be accomplished by certain catalytic substances and that in the tissue cells there are enzymes which he called oxidases which accomplish this action.

The first oxidase enzyme isolated and described by Warburg was found to consist of two types of chemical material: a protein which adsorbed the substance to be acted upon and a prosthetic group which accomplished the actual change in the molecular oxygen itself. In many of these oxidases the essential part of the prosthetic group proved to be a metal.

Warburg's picture of the process is shown in equations (5a) and (5b) and Warburg's explanation was that in such a compound the proteid part fixed the substance to be oxidized and the metal was the activator of the molecular oxygen.

Originally Warburg believed that this operation which resulted in the formation of a peroxide (X FeO<sub>2</sub>) was all that was necessary to accomplish the oxidation of a substance.

It was soon shown, however, by Wieland, that when oxidation consisted in the removal of hydrogen from a compound, it was also necessary to activate the hydrogen atoms to persuade them to let go the compound to which they were attached. Wieland also isolated enzymes that like the oxidases consisted of proteins and prosthetic groups which would accomplish this process and which are today called dehydrogenases. Wieland first demonstrated the need of a catalyst to activate hydrogen removal by use of palladium black. He showed that he could convert acetaldehyde to acetic acid by the use of this catalyst and that this process could be made continuous by providing another substance such as quinone to restore the palladium black to its condition of hydrogen acceptor. Wieland's operation is shown in equations (6a) and (6b):

Acceptor

These two concepts of oxidation in cells first requiring activation of both the hydrogen in the compound to be oxidized and of the oxygen brought to the tissue cell by the blood made necessary a new idea of what happens within the cell. According to this concept we can express the operation as shown in equations (7a) and (7b):

Catalyst

- (7a) Sugar in presence of a dehydrogenase loses 2H to a hydrogen acceptor
- (7b) Oxygen in presence of an oxidase is enabled to act as the hydrogen acceptor and receive the 2H let loose by the sugar

But this process was soon shown to be even more complicated. It was found that sugar, for example, before becoming susceptible to the action of the dehydrogenase had in certain instance to first be phosphorylated; in other words, to enter into combination with phosphoric acid. It was also found that the activated hydrogen did not usually pass directly to the activated oxygen but was transferred through a series of intermediate carriers which in turn had to be activated by specific enzymes and that the number of these carriers of hydrogen between the original sugar molecule and the ultimate delivery of hydrogen to form water with oxidase were often several in number.

A large part of the study of oxidation in recent years has therefore concerned itself with the isolation and chemical identification of these dehydrogenases, oxidases, and hydrogen carriers and the interesting viewpoint from the vitamin angle, has been the observation that certain of the vitamins form essential parts of the prosthetic groups in these enzymes and carriers.

From the energy viewpoint this somewhat complicated process of hydrogen transfer has the advantage of supplying energy in small but successive amounts rather than in a single explosive release of the energy in a single operation.

The union of two atoms of hydrogen with one of oxygen to form water if it took place all at once would liberate 68 calories of energy. By the use of intermediates such as described above, it is possible for these 68 calories to be liberated a few at a time instead of all at once, and thus provide a gradual supply of energy to the tissue cell. The phosphoric acid reactions also are a means to supplying further amounts of energy in cell metabolism.

In table 9 is shown a series of steps in the conversion of sugar to pyruvic acid. It will be seen by study of these successive steps that there is both transfer of hydrogen and of phosphoric acid from compound to compound before we arrive at the production of pyruvic acid.

#### Step 1. Glycogen or glucose is converted into Hexose-diphosphate

Fructose-diphosphate

The phosphoric acid for this process is believed derived from phosphagene by the following steps:

COOH

CH<sub>2</sub>

N—CH<sub>3</sub>

C=NH

C=NH

OH

CH<sub>2</sub>

$$+H_2O$$

N—CH<sub>3</sub>

C=NH

N—CH<sub>3</sub>

N—CH<sub>4</sub>

N—CH<sub>5</sub>

N—CH<sub>5</sub>

N—CH<sub>5</sub>

N—CH<sub>8</sub>

N—CH

Phosphagene Creatine-phosphate

Glycogen -

Creatine Phosphoric Acid

The H<sub>3</sub>PO<sub>4</sub> by union with adenylic acid forms Adenyl pyrophosphate which carries it to the sugar.

Step. 2. The next step is the scission of the hexose phosphate into two 3 carbon (triose) phosphates

Step 3. By oxidizing one molecule of phosphoglyceric aldehyde and reducing another phosphoglycerol and phosphoglyceric acid are formed. In these changes activating enzymes and hydrogen carriers are required

Step 4. In this step enzymes called mutases are involved to shift groups within the phospho-glyceric acid molecule

Step 5. Then comes another series of oxidation changes and release of phosphoric acid which resynthesizes the adenylic acid to adenyl-pyrophosphate again and permits repetition of the whole series with new sugar molecules

It will be evident from the study of this table that there is a progressive phosphorylation and hydrogen transfer starting with sugar through an intermediate series of compounds until finally pyruvic acid is produced. But this is not the end of the story.

It was early shown that if a muscle was made to contract continuously by electric current it reached a state when the current produced no further stimulation and that when this state was reached the muscle tissue had acquired a considerable quantity of lactic acid. Furthermore, no further stimulus would set the muscle contracting again until this lactic acid had been removed. It was also shown that the changes up to the formation of lactic acid could be accomplished without the presence of any oxygen and hence these stages are spoken of as stages of anerobic oxidation. The removal of the lactic acid, however, involved the use of oxygen brought to the muscle by the blood with ultimate formation of water and CO<sub>2</sub>. In the active muscle this procedure was called aerobic oxidation and shown to dispose of part of the lactic acid while part of it was resynthesized back to glycogen. How then does pyruvic acid become converted into lactic acid and into CO2 and water? There are various stages by which this can occur.

Pyruvic acid by reduction, i.e., by acceptance of two hydrogens can go over to lactic acid and this process is reversible:

(8) 
$$C \longrightarrow O$$
 $C \longrightarrow OH$ 
 $C$ 

It has also been shown that in yeast fermentation pyruvic acid can be de-carboxylated (have its CO<sub>2</sub> split out by carboxylase enzyme) and can become converted to acetaldehyde and that in turn, by oxidation, converted to acetic acid and ultimately to CO<sub>2</sub> and water.

It is of interest to note in this reaction that the  $CO_2$  obtained does not come from the union of oxygen with a carbon atom in the molecule of pyruvic acid but is actually obtained by splitting the  $CO_2$  out of the pyruvic acid. This fact has a special interest to the vitamin student because it is now known that vitamin  $B_1$  plays a definite rôle in cooperating with a carboxylase to accomplish this  $CO_2$  extraction. In the human body, however, there is evidence that the conversion of pyruvic acid to  $CO_2$  and water involves the formation of other inter-

mediates than those which occur in the yeast fermentation, among them products like succinic acid:

Active work is now going on to determine just what these intermediates are, with evidence that citric acid may be involved in the process.

The main importance of the steps pictured in the preceding tables and equations is to point out the fact that hydrogen transfer is a fundamental operation in the release of energy from body fuel; and that for this operation dehydrogenases, oxidases, hydrogen acceptors and hydrogen carriers as well as carboxylases, phosphatases, and mutases are involved.

Let us now consider some of the actual enzymes and substances that have been found to accomplish these changes and their relation to vitamin molecules.

#### COENZYMES

Some years ago it was shown that yeast contained a ferment or enzyme which would convert glucose to alcohol. This enzyme was called zymase. What was originally known as zymase was probably a collection of enzymes rather than a single one but it was later shown that this zymase in enzyme combination alone did not accomplish complete conversion

of the glucose to alcohol. There had to be present a heat stable substance different from the zymase to complete the transformation. This substance was called a cozymase or coenzyme.

Today, two such coenzymes have been isolated and identified chemically. The one that was associated with the zymase and originally called cozymase is now known as coenzyme I and a second one has been isolated and its structure determined. It is called coenzyme II. The structure of coenzyme II is shown below:

and coenzyme I is supposed to be similar but with only two

phosphoric acid groups.

There are several points of interest to this formula. The student of biochemistry will at once recall that the protein which characterizes the nucleus of cells has been shown to contain a series of nucleotides which in turn are composed of phosphoric acid, a sugar, and a purine or pyrimidine base (see table 10).

A glance at coenzyme II's formula will show that it is in essence a nucleotide with a purine base (adenine) linked to phosphoric acid by the sugar d-ribose. Another interesting feature is that in coenzyme II, there is another group also linked to phosphoric acid by the ribose sugar which is identical with what we now know as the antipellagric vitamin, namely, nicotinic acid amide.

#### TABLE 10

What does the presence of this nicotinic acid amide in the coenzyme supply for the process of oxidation? To answer that question, we need another equation. Nicotinic acid amide can act as a hydrogen acceptor and this reaction is reversible. The ability to make this change is due to the presence in the nicotinic acid or amide of what the chemist knows as the pyridine ring (see table 11).

It is of further interest to note that not only is the pyridine ring present in the anti-pellagra vitamin, nicotinic acid and amide, but that it is also present in the vitamin that is known today as pyridoxin or vitamin  $B_0$ . Both nicotinic acid, the

pellagra-preventive vitamin, and pyridoxin marked changes in epithelial tissues. In view of the fact that they are parts of the prosthetic group of hydrogen accepting coenzymes it is obvious that the epithelial changes may be due to lack of enough of these substances to build coenzyme and with lack of coenzyme there results a disturbance of metabolism within the tissue cells.

#### TABLE 11

Nicotinic Acid Amide

Pyridine Ring

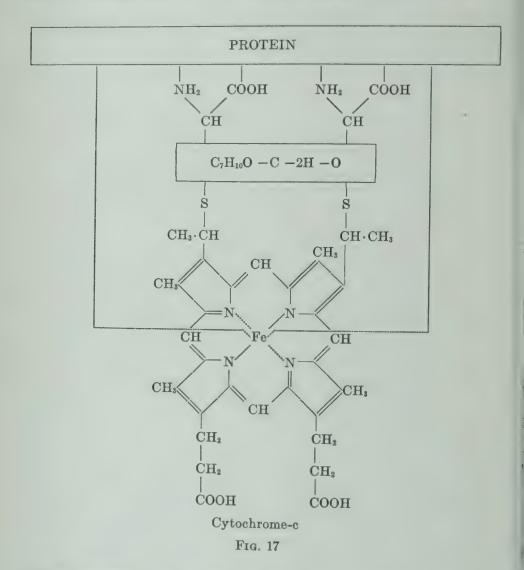
Reduced Nicotinic Acid Amide

Before leaving consideration of the coenzyme structure note also that the adenyl fraction makes it possible for that coenzyme to also supply material for the manufacture of adenyl pyrophosphate which we have seen is intimately associated with the oxidation-reduction process as a carrier of phosphoric acid.

#### CYTOCHROMES

Warburg's original oxygen activating enzyme or oxidase came later to be called the indophenol oxidase because when

combined with dimethyl-p-phenylene diamine and alphanaphthol in the presence of oxygen a blue dye known as indophenol was produced. The same dye substance is used to



oxidize ascorbic acid. Today it is better described as cytochrome oxidase because we now know that there is a series of three compounds, cytochromes A, B, and C which have to be activated by this oxidase to permit the transfer of electrons which they accept from hydrogen to the oxygen atoms. What are these cytochromes? The structure of cytochrome C as worked out by Theorell is shown in figure 17.

Cytochromes were first discovered some years ago by MacMunn and called by him "myohematins." They were rediscovered by Keilin who first showed them to be not only widely distributed in cells but concerned in respiration. A glance at the diagram will show two outstanding structures present, namely, a protein group attached to metallic iron in the same sort of linkage (tetra-pyrrol) as is found in blood hemoglobin.

It is the iron in these cytochromes which permits the transfer of electrons from hydrogen and ultimately, under the influence of Warburg's oxidase, to oxygen.

Szent-Györgyi reports the process to take place as follows:

The substrate or hydrogen donor does not actually give hydrogen atoms to cytochrome B but these hydrogen atoms pass over to it their negative electrical charge, which in turn is taken up by the iron. As a result of this electron transfer, the iron is transformed from ferric to ferrous state (Fe<sup>+++</sup>  $\rightarrow$  Fe<sup>++</sup>) and the hydrogen, bereft of its electron, becomes a hydrogen ion (H<sup>+</sup>). Cytochrome B then gives up its recently acquired electrons to cytochrome C, and that in turn to cytochrome A and this passes it on to the oxygen atom. When the oxygen atom in this way has acquired 2 electrons, from 2 hydrogen atoms, it becomes an oxygen ion (O<sup>--</sup>). Two hydrogen ions and one oxygen ion then proceed to combine into a molecule of water. The cytochromes with their iron electron acceptor activated by the cytochrome oxidase therefore actually pass the electrons from hydrogen to oxygen and it is this transfer that makes the formation of water possible through the interaction of the ionized hydrogen and oxygen.

#### **DEHYDROGENASES**

Primarily a dehydrogenase is an enzyme system and all these enzyme systems depend on a specific adsorptive agent to fix the substance which is to be acted upon, protein being the usual type of adsorbent. Specificity is also determined by the absorptive agent, certain substrates being specifically adsorbed by particular proteins.

In classifying either dehydrogenases or oxidases therefore, a given enzyme system may use a specific protein in combination with coenzymes and other hydrogen carriers. On this basis the following classification of dehydrogenases has been proposed by Linderstrom-Lang:

Group I. Anaerobic dehydrogenases

- (a) X ~ pyridine carrier ~ flavoprotein dye
- (b) X ~ pyridine carrier ~ dye

(c) X ~ pyridine carrier ~ Y Group II. Aerobic dehydrogenases

- (a) X  $\sim$  pyridine carrier flavoprotein cytochrome  $\sim$  O<sub>2</sub> (b) X  $\sim$  pyridine carrier flavoprotein O<sub>2</sub> or H<sub>2</sub>O<sub>2</sub> formed

X = Substrate. Y = Oxidized Product.

~ indicates coenzymes or carriers cooperating.

"—" means no other enzyme.

For example, the typical dehydrogenase reaction could be written as follows:

- 1. Hexose mono-phosphate in the presence of a dehydrogenase loses 2 hydrogens which pass to a coenzyme.
- 2. The coenzyme passes its hydrogen to a yellow enzyme whose structure is such as we showed on p. 24.
- 3. The yellow enzyme passes the 2 hydrogens or their electrons to cytochromes.
- 4. The cytochromes pass the 2 hydrogens or the electrons on to molecular oxygen which either forms a peroxide or becomes ionized and is thus enabled to react with the ionized hydrogen produced when those hydrogen atoms give up their electrons to cytochrome.

Oxidases then are similar to dehydrogenases in that they too are combinations of proteins for specific adsorption with hydrogen or electron acceptance factors.

It has already been shown that Warburg's yellow enzyme or flavin-protein is not the only flavin-protein dehydrogenase combination and a recent discovery is such a flavin-protein identified as "diaphorase."

#### SUMMARY

The enzymes, coenzymes, flavo-proteins, etc., involved in the transport of hydrogen from substrate to its ultimate acceptance by oxygen is of particular interest to the study of vitamins for the reason that several of these vitamins have today been shown to contain chemical groups which can act as hydrogen acceptors. If then, the diet fails to provide these groups it is obvious that the tissue cells can not build the coenzymes and enzymes necessary for the oxidative processes and that a further consequence of this will be that the oxidative process in the tissue cell will be blocked to a greater or lesser degree and cell respiration interfered with. Because of that it also becomes evident that in any given tissue cell such failure of tools for carrying out metabolism of the cells' foodstuff must definitely interfere with the performance of such cells.

Vitamins therefore become essential to cell behavior and normality. The results of such abnormality in vitamin deficiency may manifest themselves in a great variety of ways. The symptom by which the physician recognizes the existence of such deficiency may be in a region of the body quite removed from the place where the deficiency actually interferes with the cell behavior and a major problem of the clinician today is to learn to recognize the symptoms and the location of basic injury.

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### CHAPTER IV

# VITAMIN REQUIREMENTS

The problem of vitamin needs has at least two aspects. In choosing the daily diet it is important that there shall be sufficient of these factors in kind and amount to prevent the development of deficiency disease. What are the sources of the vitamins, what is the potency of these sources, what are reasonable allowances to make in planning diets, are therefore matters of public concern.

To the physician faced with the correction of vitamin deficiency disease the amounts necessary for correction and a means of measuring the effect of his dosages are of paramount importance. What data are available today for these two

sorts of requirements?

In pages 000–000 we have tabulated the approximate vitamin A, B<sub>1</sub>, C and B<sub>2</sub> potencies of some of our common foodstuffs per 100 grams (3.5 ozs.) of fresh raw material. These data have been culled from published assays of such foods. They do not, of course, represent the values of actually ingested foods as affected by methods of cooking or methods of storage and merchandising which may and often do seriously reduce the values present in the fresh raw products. They do, however, at least indicate the relative potency of different sources of vitamins and the maximum amounts to be secured by selection of such sources.

Based on such data combined with dietary surveys correlated with evidences of vitamin deficiency several reports are today available indicating probable adequacy or inadequacy of diets at different budget levels. One of these by J. B. Orr covering conditions in Great Britain has been revelatory of possible

deficiency in different budget groups in that country. Table 12 gives some of the data presented by Orr.

By study of the foods purchased by these groups, estimate of their vitamin potency, and a table of requirements suggested by Dr. Hazel Stiebeling of the U. S. Department of Agriculture, Orr drew the conclusion that in Group I the diet was inadequate in all the constituents studied; Group II adequate only in protein and fat; Group III adequate in energy foods, protein and fat but below standard in minerals and vitamins; Groups

TABLE 12

Budget Groups of English Population
(After J. B. Orr)

| GROUPS  | INCOME PER<br>PERSON PER WEEK | ESTIMATED AVERAGE FOOD EXPENDITURE PER PERSON PER WEEK | ESTIMATED POPULATION OF GROUP | PER CENT OF<br>POPULATION |
|---------|-------------------------------|--|-------------------------------|---------------------------|
|         | shillings                     | shillings  |                               |                           |
| I       | Up to 10                      | 4  | 4,500,000                     | 10                        |
| II      | 10-15                         | 6  | 9,000,000                     | 20                        |
| III     | 15-20                         | 8  | 9,000,000                     | 20                        |
| IV      | 20-30                         | 10   | 9,000,000                     | 20                        |
| V       | 30-35                         | 12   | 9,000,000                     | 20                        |
| VI      | Over 35                       | 14   | 4,500,000                     | 10                        |
| Average | 30                            | 9  |                               |                           |

IV and V adequate in all but calcium and only Group VI exceeding requirements in all particulars.

If the requirement data and vitamin potency figures are valid Orr's survey indicates probable vitamin deficiency in the diets of at least 50 per cent of the English population.

WHAT ARE THE STIEBELING SUGGESTED REQUIREMENTS?

There has been published from the Home Economics Department of the U.S. Department of Agriculture a Circular (507) by Stiebeling and Phippard. This is a report of a dietary survey similar to that of Orr covering certain classes

TABLE 13
(From Circular \*50; Dept. Home Econ., U. S. Dept. Agric.) Stiebeling and Phippard

| TYPE OF INDIVIDUAL             | AVER-  | AVER-  | CALO-<br>RIE | SUG  |                | DAILY NE<br>AMINS | ED OF        |
|--------------------------------|--------|--------|--------------|------|----------------|-------------------|--------------|
|                                | HEIGHT | WEIGHT | NEED         | A    | B <sub>1</sub> | C                 | B2(G)        |
|                                | in.    | lbs.   |              | i.u. | i.u.           | 1.16.             | S-B<br>units |
| Men, 20-59 years of age        | 68     | 154    |              |      |                |                   |              |
| Mod. active work               |        |        | 3000         | 6000 | 500            | 1500              | 600          |
| Very active work               |        |        | 4500         | 6000 | 500            | 1500              | 600          |
| Light work                     |        |        | 2700         | 6000 | 500            | 1500              | 600          |
| Sedentary work                 |        |        | 2400         | 6000 | 500            | 1500              | 600          |
| Women, 20-59 years of age      | 64     | 132    |              |      |                |                   |              |
| Mod. active work               |        |        | 2500         | 6000 | 500            | 1500              | 600          |
| Very active work               |        |        | 3000         | 6000 | 500            | 1500              | 600          |
| Light work                     |        |        | 2300         | 6000 | 500            | 1500              | 600          |
| Sedentary work                 |        |        | 2100         | 6000 | 500            | 1500              | 600          |
| Boys, 4-19 years of age:       |        |        |              |      |                |                   |              |
| 16-19 years                    | 68     | 139    | 3600         | 6000 | 600            | 1800              | 600          |
| 13-15 years                    | 63     | 111    | 3000         | 6000 | 500            | 1500              | 600          |
| 11-12 years                    | 57     | 82     | 2500         | 6000 | 420            | 1350              | 600          |
| 9-10 years                     | 53     | 68     | 2400         | 5400 | 400            | 1200              | 540          |
| 7- 8 years                     | 49     | 55     | 2100         | 5400 | 350            | 1000              | 540          |
| 4- 6 years                     | 42     | 40     | 1500         | 4500 | 250            | 1000              | 450          |
| Girls, 4-19 years of age:      |        |        |              |      |                |                   |              |
| 14-19 years                    | 64     | 121    | 2500         | 6000 | 420            | 1350              | 600          |
| 11-13 years                    | 58     | 89     | 2400         | 5400 | 400            | 1200              | 540          |
| 8–10 years                     | 52     | 64     | 2100         | 5400 | 350            | 1000              | 540          |
| 4- 7 years                     | 42     | 39     | 1500         | 4500 | 250            | 1000              | 450          |
| Children under 4 years of age: |        |        |              |      |                |                   |              |
| 2-3 years                      | 25     | 90     | 1000         | 4500 | 000            |                   |              |
| Under 2 years.                 | 35     | 29     | 1200         | 4500 | 200            | 1000              | 450          |
| Judis Judis                    | 30     | 22     | 900          | 4500 | 200            | 1000              | 450          |

N.B.: One International Unit (I.U.) of A=0.0006 mgm. carotene (beta); one International Unit (I.U.) of  $B_1=0.003$  mgm. thiamin; one International Unit (I.U.) of C=0.05 mgm. ascorbic acid.

One Sherman-Bourquin Unit  $B_2(G)$  or Riboflavin = 0.003 mgm. riboflavin.

of the population of the United States and giving suggestion as to nutrient needs for certain age, sex and occupation groups. The data as related to vitamin A, B<sub>1</sub>, C, and G (B<sub>2</sub>) requirements we have summarized in table 13.

What is the basis for these requirements suggested by Stiebeling and Phippard?

In 1938, the Journal of the American Medical Association published a series of articles covering the problem of requirement for vitamins A, B<sub>1</sub>, C, D, and G with a review of existing data for forming estimates of needs. The findings of these reviewers may be summarized as follows:

# Vitamin A (Booher, 1938)

Booher considers the collective evidence to justify the view that an amount of vitamin A just sufficient to prevent hemeralopia in an adult is 20 to 30 I.U. per kilo of body weight or 1400 to 2000 units daily for a 70 kilo (150 lb.) adult. She suggests 6000 to 8000 units daily for the growing child to cover both maintenance and growth developments, and for pregnant or lactating women 5000 units daily.

Since hemeralopia appears to be the earliest manifestation of vitamin A deficiency the amounts necessary to prevent this condition have received especial attention but unfortunately there is considerable difference of opinion as to the accuracy of these various dark adaptation measurements (see Chapter VI). Jeghers, for example, using medical students as subjects reached the conclusion that 4000 units was the minimum requirement to insure prevention of hemeralopia and suggested a 50 per cent factor of safety or 6000 units per day which is Stiebeling's recommendation. Jeans, Blanchard and Zentmire found 3000 units per day, by photometer tests, adequate for 11 year old boys, a figure considerably under Stiebeling's recommendation.

In general from review of all data available, it would appear that Stiebeling's estimates allow a fair margin of safety for the types cited assuming normal health. It is quite possible that smaller amounts may suffice but since there are no toxic effects of larger doses of this vitamin it seems best to use larger factors of safety rather than amounts too close to minimum needs.

Sherman is an advocate of generous intakes of vitamin A to insure what he calls optimum in contrast to merely average normal nutrition, e.g.:

The evidence now at hand in the case of vitamin A, indicates that those dietaries giving the best results are those whose vitamin A values are several-fold higher than the intakes which would just cover the readily demonstrable actual need. To obtain the fullest measure of benefit, in the experiments of Mellanby and Green, requires four times the intake of vitamin A value which sufficed to support normal growth and every appearance of normal health. In Batchelder's investigation of the influence of the different levels of vitamin A feeding upon the nutritive welfare of experimental animals throughout their lives and into the second generation, it appeared that the best all round results were obtained only when the vitamin A value of the food was four times as high as was demonstrably necessary to support normal growth and all the visible indications of good health.

Rose recommends 200 Sherman Units per 100 calories of food intake for children and 100 per 100 calories for adults. The Sherman Unit is considered today as equivalent to 1.4 International Units. On that basis Rose's recommendations would compare with Stiebelings as shown in table 14.

There is another argument for liberal rather than restricted figures for vitamin intake in that the principal food source of vitamin A (vegetables) in the ordinary diet provides the factor as carotene rather than active vitamin A.

Guilbert, Miller and Hughes studied the vitamin needs of three species of animals (cattle, sheep, and swine) and found a remarkable agreement in requirement when expressed in units per kilo of body weight. They used prevention of hemerolopia as the criterion of adequacy. They suggested that the minimal requirement of man on this basis calls for 40 to 50 units per kilo of body weight if carotene is the source and 20 to 30 units per kilo if active A is used. How these

compare with Stiebeling's estimates is also shown in table 14. It will be noted that even adding a 50 per cent factor of safety to the Guilbert estimates they are less liberal than Stiebeling's even when the figures for carotene are used.

TABLE 14

| GUILBERT MI | LLER HUGHES<br>DATION FOR |       |      |                 | CALORIE | STEIBEL-<br>ING RE- | ROSE<br>SUG-<br>GESTED |
|-------------|---------------------------|-------|------|-----------------|---------|---------------------|------------------------|
| Carotene    | Vitamin A                 | WE    | GHT  | TYPE INDIVIDUAL | INTAKE  | QUIRE-<br>MENT      | RE-<br>QUIRE-<br>MENT  |
|             |                           | kilos | lbs. |                 |         |                     |                        |
| 2800-3500   | 1400-2100                 | 70    | 154  | Adult man       | 3000    | 6000                | 2130                   |
| 2400-3000   | 1200-1800                 | 60    | 132  | Adult woman     | 2500    | 6000                | 1775                   |
|             |                           |       |      | Boys            |         |                     |                        |
| 2520-3150   | 1260-1990                 | 63    | 139  | 16-19           | 3000    | 6000                | 5148                   |
| 2000-2500   | 1000-1500                 | 50    | 111  | 13–15           | 3000    | 6000                | 4290                   |
| 1480-1850   | 640-1100                  | 37    | 82   | 11-12           | 2500    | 6000                | 3575                   |
| 1240-1550   | 620-930                   | 31    | 68   | 9–10            | 2400    | 5400                | 3432                   |
| 1000-1250   | 500-750                   | 25    | 55   | 7–8             | 2100    | 5400                | 3003                   |
| 720-900     | 360-540                   | 18    | 40   | 4-6             | 1500    | 4500                | 2145                   |
|             |                           |       |      | Girls           |         |                     |                        |
| 2200-2750   | 1100-1650                 | 55    | 121  | 14–19           | 2500    | 6000                | 3575                   |
| 1600-2000   | 800-1200                  | 40    | 89   | 11-13           | 2400    | 5400                | 3432                   |
| 1160-1450   | 580-870                   | 29    | 64   | 8–10            | 2100    | 5400                | 3003                   |
| 720-900     | 350-540                   | 18    | 39   | 4-7             | 1500    | 4500                | 2145                   |
|             |                           |       |      | Children        |         |                     |                        |
| 520-650     | 260-390                   | 13    | 29   | 2-3             | 1200    | 4500                | 1716                   |
| 400-500     | 200-300                   | 10    | 22   | Under 2         | 900     | 4500                | 1287                   |

In general then the Stiebeling estimates appear to be quite safe estimates for prevention of A deficiency symptoms whatever the source.

# $Vitamin B_1 (Cowgill, 1938)$

Cowgill has derived a formula for expressing the minimum B<sub>1</sub> needs of the individual provided one has the calorie intake and weight in kilograms. This formula in International Units is as follows:

Vitamin Need in I.U. =  $.00142 \times Wt$ . in kilos  $\times$  Calorie intake

# Of this formula estimate Cowgill says:

It should be emphasized that estimates of the human requirement for vitamin B derived from my formula pertain to the minimum or beri-beri preventing level; the optimal intake is undoubtedly much greater.

TABLE 15

|                    |       | IADUE | 10    |                | 1             |                            |
|--------------------|-------|-------|-------|----------------|---------------|----------------------------|
|                    |       |       |       | STIEBEL-       | COWGILL A     | LLOTMENT:                  |
| TYPE OF INDIVIDUAL | WE    | ІСНТ  | NEEDS | ALLOW-<br>ANCE | By<br>formula | + 50 per<br>cent<br>factor |
|                    | kilos | lbs.  |       |                | i.u.          |                            |
| Man adult          | 70    | 154   | 3000  | 500            | 298           | 447                        |
| Woman adult        | 60    | 132   | 2500  | 500            | 213           | 314                        |
| Boy:               |       |       |       |                |               |                            |
| 16-19 years        | 63    | 139   | 3000  | 600            | 321           | 481                        |
| 13-15 years        | 50    | 111   | 3000  | 500            | 213           | 314                        |
| 11-12 years        | 37    | 82    | 2500  | 420            | 131           | 186                        |
| 9–10 years         | 31    | 68    | 2400  | 400            | 105           | 158                        |
| 7–8 years          | 25    | 55    | 2100  | 350            | 75            | 112                        |
| 4-6 years          | 18    | 40    | 1500  | 250            | 38            | 47                         |
| Girl:              |       |       |       |                |               |                            |
| 14-19 years        | 55    | 121   | 2500  | 420            | 195           | 293                        |
| 11–13 years        | 40    | 89    | 2400  | 400            | 136           | 204                        |
| 8–10 years         | 29    | 64    | 2100  | 350            | 87            | 131                        |
| 4-7 years          | 18    | 39    | 1500  | 250            | 38            | 47                         |
| Children:          |       |       |       |                |               |                            |
| 2-3 years          | 13    | 29    | 1200  | 200            | 22            | 33                         |
| Under 2 years      | 10    | 22    | 900   | 200            | 13            | 20                         |

In table 15 we have calculated Cowgill's suggestions for minimum requirements based on his formula, the amounts using a 50 per cent factor of safety, and their comparison with the Stiebeling recommendations.

It is evident from this comparison that for adults the Stiebeling figures provide a satisfactory allowance for safety and a very liberal margin for young children. That such

liberality for younger children is justified is shown by other studies in the lower age groups.

Knott, working with children 4 to 7 years of age, found best results with 20 units per kilo daily or 20 to 25 units per 100 calories of intake. This would figure for 4 to 7 year old children at 360 daily on weight basis or 300 to 375 daily on calorie basis.

In infants mainly on milk feedings it is particularly essential that B<sub>1</sub> supplements be given as milk is not rich in this factor (ave. cow's milk: 100 to 250 units B<sub>1</sub> per quart).

The following estimates of adult needs have been cited by

the observers listed:

Jansen—200 daily or 10 per 100 calories.

Rose—15 per 100 calories = 375 to 450.

Vorhaus, Williams, Waterman—333.

Harris and Leong—at least 200.

Van Veen—150 units represents the minimum protective dose.

Baker and Wright—200 to 500.

Stepp—333 to 666.

Cowgill's own suggestion is 15 to 20 units per 100 calories. It is generally agreed, however, that following a period of deficiency much more of B<sub>1</sub> per day is necessary to initiate recovery.

# Vitamin C (Smith, 1938)

Thanks to criteria such as measurement of dosage necessary to saturate tissues as shown by urinary analyses and blood values we have better indices of requirement for this vitamin than for some of the other types. Smith has reviewed such data and presents the following as lying within the limits of adequate values:

Infants from 8 to 50 mgm. daily (160 to 1000 units)
Children from 22 to 100 mgm. or more daily (440 to 2000 units)
Adults from 28 to 100 mgm. or more daily (416 to 2000 units)

The lower value represents the indispensable minimum and

the upper value probably an optimum allowance.

She considers that C requirement is influenced by both weight and metabolic rate and the latter more pronounced in effect. On the basis of weight the following values have been suggested:

Infants 3 to 8 mgm. per kilo body weight (60 to 160 units) Children 6.4 to 7.5 mgm. per kilo body weight (128 to 150 units) Adults 0.7 to 1.6 mgm. per kilo body weight (14 to 32 units)

On this latter basis the estimates compare with Stiebeling's suggestions as shown in table 16.

It is obvious from this comparison, even using the smaller estimates that the allowances cited by Smith are far more liberal than those in the Stiebeling series. Are the latter too low?

More recently Schroeder has stated the adult requirement

at 50 mgm. per day (1000 units).

In estimating vitamin C needs, two criteria have been in use now for several years. One has been based on the theory that if a person is given a massive dose either by mouth or intravenously the excretion of less than 50 per cent of that dose in the urine in the next twenty-four hours indicates a lack of saturation of the tissues. To produce satisfactory indication of saturation by this means and to maintain a normal urinary secretion have therefore been the basis for prescribing requirements.

In the earlier use of this method Harris and his associates reached the conclusion that if a subject failed to excrete more than 13 mgm. of ascorbic acid in the urine daily and failed to show a marked increase in excretion on the first or second day following a 700 mgm. test dose per 140 lbs. body weight, the diet was not adequate in C. Using similar methods van Eekelen set an excretion in urine of 40 mgm. per day as indicating a state of saturation.

This variation in viewpoint makes it obvious that the

urinary excretion method is far from satisfactorily established and this is due in part to the imperfection in the test itself to date. Tests on this basis have also shown quite wide variation in individual responses to the same C intake.

Widenbauer developed a urinary excretion method for determining minimum requirements that was in brief as follows: In a preliminary period of several days with a uniform diet

TABLE 16

| TYPES OF INDIVIDUALS | WEIGHTS    | STIEBELING<br>ALLOWANCES | SMITH ALLOWANCES |
|----------------------|------------|--------------------------|------------------|
|                      | kilos      |                          |                  |
| Adult man            | 70         | 1500                     | 980-2240         |
| Adult woman          | 60         | 1500                     | 840-1920         |
| Boy:                 |            |                          |                  |
| 16–19                | 63         | 1800                     | 8064-9450        |
| 13–15                | 50         | 1500                     | 6400-7500        |
| 11-12                | 37         | 1350                     | 4736-6550        |
| 9-10                 | 31         | 1200                     | 3968-4650        |
| 7-8                  | 25         | 1000                     | 3200-3750        |
| 4-6                  | 18         | 1000                     | 2304–2700        |
| Girl:                |            |                          |                  |
| 14-19                | 55         | 1350                     | 7040-8250        |
| 11–13                | 40         | 1200                     | 5120-6000        |
| 8-10                 | <b>2</b> 9 | 1000                     | 3712-4350        |
| 4-7                  | 18         | 1000                     | 2304-2700        |
| Infants:             |            |                          |                  |
| 2–3                  | 13         | 1000                     | 780-2080         |
| Under 2              | 10         | 1000                     | 600-1600         |

low in C, the titration value of the urine was determined on two 12 hour samples and the average daily value obtained, this to be deducted from the final average excretion. Test doses of 200 to 500 mgm. ascorbic acid per day were then given orally until saturation was indicated by at least 50 per cent of the test dose being excreted in 12 hours. With the individual saturated there follows a period of adjustment of daily doses to the amount necessary to give an excretion value slightly higher than that of the first period on the low C diet before saturation. When this dosage is established the same quantity is given for a seven day period and the average daily excretion of this period established.

The final calculation of requirement is made by subtracting from the average daily intake during the final period the average daily excretion of the same period minus the average daily excretion of the preliminary period. By this method Widenbauer established as his own requirement and the requirement of a non-pregnant woman over a period of 210 days, 28 mgm. of ascorbic acid or 560 units daily. On the other hand van Eekelen, by another method, reached an estimate of her need as 63 mg. daily or 1260 units and in two other studies by essentially van Eekelen's method requirements of 0.7, 0.78, 0.83, and 0.83 mgm. per kilo of body weight were obtained for 4 adults. For the adult of 60 kilos weight the range in requirement was 42 to 50 mgm. daily or nearly twice the minimum established by the Widenbauer method.

Another method of establishing daily C need extensively used by Göthlin was the determination of the amount of daily intake of vitamin C necessary to maintain normal capillary resistance. This condition was reached on daily intakes of 0.7 to 1 cc. of orange juice per kilo of body weight or 21 to 30 mgm. ascorbic acid for an adult weighing 60 kilos (132 lbs.). Later he checked these results using pure ascorbic acid and got good agreement, viz., values of 19 to 27 mgm. daily and still later reached estimates of 23.4 to 28.8 for an adult of 60 kilos weight. Göthlin claims this method establishes the physiologically indispensable daily need.

Farmer and Abt developed a technique for measuring the reduced ascorbic acid content of the blood and this and other methods have been extensively used to establish what appears today to be satisfactory indices of adequate C intake and

utilization. In brief a blood plasma content of 1 mgm. ascorbic acid or more per 100 cc. is considered evidence of adequate supply and absorption. Similarly a blood content of 0.5 mgm. per 100 cc. or less is indicative of a scorbutic status. Intermediate amounts indicate what is known as the prescorbutic stage. It would be better to consider that the low values indicate degrees of depletion predisposing to scurvy.

Use of this method and its correlation with capillary resistance and urinary excretion methods have already been

somewhat extensively studied.

Braestrup found that initially low blood values of infants could be raised from 0.56 to 0.76 mgm. per cent by daily doses of 20 mgm. ascorbic acid but were not affected by 10 mgm. doses. This would mean that in the first days after birth infants should receive at least 20 mgm. of ascorbic acid or 5 mgm. per kilo of body weight. Neuweiler set the figure for very young infants at 6 mgm. per kilo of body weight.

For children we have few data correlated with blood values. Conclusions are generally based on urine studies and the range is wide; from 20 to 21 mgm. as minimum requirement for a 3 year old by the Widenbauer method to minimum saturation values of 117 to 123 mgm. for children of 3 to 5 years by the methods of Everson and Daniells. Hamil and assocates state that studies with 427 children indicated the minimum protective dose to be 10 mgm. per day.

For adults we also have lack of satisfactory data correlating intake with effect on blood C. Mackie and Eddy examined the blood plasma of 88 apparently normal individuals with no signs of scurvy. Twenty-five per cent of these individuals, however, were found to have a plasma content of less than 0.5 mgm. per cent, the average of the other 63 was 1.06 mgm. with a standard deviation of 0.37 mgm. per cent. Farmer has reported a case receiving daily 1000 mgm. of ascorbic acid with the following effects on blood C and excretion.

It is evident from these data that if blood C at 1 mgm. per cent or over is evidence of tissue saturation there is a surprising

TABLE 17

| DATES   | BLOOD C       | URINARY C-24 HOURS         | FECAL C-24 HOURS        |
|---------|---------------|----------------------------|-------------------------|
|         | mgm. per cent |                            |                         |
| Oct. 12 | 1.42          | 630 mgm. in 1560 cc. urine |                         |
| 13      | 1.01          | 531 mgm. in 1160 cc. urine | 0.94 mgm. in 23.3 grams |
| 14      | 1.50          | 281 mgm. in 1845 cc. urine |                         |
| 15      | 1.05          | 111 mgm. in 1480 cc. urine |                         |
| 16      |               | 406 mgm. in 1800 cc. urine |                         |
| 17      | 1.05          | 566 mgm. in 442 cc. urine  | 1.7 mgm. in 55 grams    |
| 18      | 0.97          | 385 mgm. in 1810 cc. urine | 1.04 mgm. in 68 grams   |
| 19      | 1.27          | 626 mgm. in 1340 cc. urine | 0.25 mgm. in 38.6 grams |

| Group I. | Individuals         | with blood C 0.5 mgm. per cent or les |
|----------|---------------------|---------------------------------------|
|          | Number of petechiae | Blood C in mgm. per cent              |
|          | 35                  | .001                                  |
|          | 25                  | .04                                   |
|          | 30                  | .05                                   |
|          | 24                  | .07                                   |
|          | 16                  | .08 (Ave. 3 cases)                    |
|          | 26                  | .09                                   |
|          | 8                   | .14                                   |
|          | 5                   | .16                                   |
| Group II | . Individuals       | with blood C above 0.5 mgm. per cen-  |
|          | 4                   | 0.6                                   |
|          | 17                  | 1.72                                  |
|          | 7                   | 2.10                                  |
|          | 14                  | 2.8                                   |
|          | 5                   | 2.97                                  |
|          | 3                   | 3.5                                   |
|          | 7                   | 3.85                                  |
|          | 2                   | 4.5                                   |

failure of correlation with urinary excretion, not accounted for by fecal loss.

Mackie and Eddy also studied correlation between blood

and capillary resistance tests with individuals showing greater and lesser values than 0.5 mgm. per cent. In those tests the number of petechiae at a constant negative pressure was taken as the criterion of fragility rather than the minimum pressure required to produce hemorrhage.

These results show that with low blood C there is correspondingly low capillary resistance but at values above 1 mgm. per cent the capillary resistance test may be quite misleading.

Sloan concluded that the capillary resistance test in the majority of cases gives dependable information concerning presence or absence of vitamin C depletion but does not satisfactorily indicate partial degrees of depletion and gives falsely negative results in the presence of severe anemia.

It is obvious from this brief review that until methods of establishing Vitamin C needs are better standardized estimates of requirements are bound to vary according to the methods used. There appears to be, however, a tendency to be liberal rather than to keep too close to minimum protective needs.

# Vitamin D (Jeans and Stearns, 1938)

Jeans and Stearns summarize their conclusions as follows:

The vitamin D requirement of the full term artificially fed baby is probably between 300 and 400 units per day.

Normal babies receiving human milk require less D than do babies receiving cow's milk, but how much less is not known. However, vitamin D is necessary for many and useful for most breast fed babies. It would seem wise to prescribe for them the same amount as is required by artificially fed babies.

It is tentatively considered that prematurely born babies may require twice as much D as full term babies during the early period of most rapid growth, after which time the requirement should be the same as for babies born at term.

For children between infancy and adolescence a daily allowance of at least 750 cc. of milk together with from 300 to 400 units of vitamin D permits consistently ample retention of calcium and phosphorus. The optimal

quantity of vitamin D cannot be stated accurately, though it appears probable that the total quantity needed is neither greater nor less than the amount required for the infant.

For adolescents a need for vitamin D exists, but insufficient data are available to permit an estimate of the quantity required. It seems probable that

from 300 to 400 units a day would be satisfactory.

For adults the optimal amount of vitamin D, if a need exists, remains to be determined.

It appears strongly advisable to give vitamin D during pregnancy and lactation. The optimal amount is not known. During lactation the requirement may be greater than at any other period of life and a daily dosage of 800 units or more is suggested together with an abundant intake of calcium and phosphorus.

In a subsequent discussion Jeans suggested 300 to 400 units as a recommendation for the adult, hence his recommendations all condense to 300 to 400 units daily for man, woman and child and double that for the pregnant and lactating woman.

Provision for vitamin D needs is markedly different than for most of the other vitamins since while its distribution in natural foods is very limited its production in the body is possible by exposure to the ultra-violet rays of the sun. Furthermore its function is conditioned by the amount of calcium and phosphorus ingested for without these elements it is of no value. Requirement is greatly increased by rate of growth.

There is also evidence that when fortified or concentrated sources are used the amounts necessary vary with the vitamin source.

Again, while for the prevention or cure of rickets in infants we have definite means of measuring necessary unitage; for adolescent and adult needs we have little to guide us. Our evidence suggests need but does not give quantitative requirements. For such groups perhaps our best index to date is the influence on calcium retention.

Jeans defines the requirements of vitamin D to be "the amounts which, with ample intakes of calcium and phosphorus and a diet otherwise adequate, insure sufficient retention of calcium and phosphorus to permit (a) normal growth and

mineralization of the skeleton and teeth for infants and children, (b) maintenance of bony and dental structures during adult life, and (c) a sufficient supply for mother and infant during pregnancy and lactation.

Calcium retention requirements have been put at from 0.1 gram daily throughout childhood to a graded scale calling for 0.3 gram at 1 year to 0.5 gram daily during adolescence. For adults metabolic equilibrium may be used.

Park has reviewed the dosages of therapeutic sources of vitamin D necessary for treatment and prevention of rickets and for the human subject states that rat unit for rat unit the different sources may be considered equivalent in practically all types. However, he considers D dispersed in milk more effective than in oil menstruums and that in the latter 800 to 1000 units rather than 400 be the lowest amount to administer daily to infants after the fourth or fifth week. With cod liver oil he suggests ½ teaspoonful (200 units) the third and fourth week; after 2 or 3 days increase to 1 teaspoon (400 units). During the 4th or 5th week the dose should be raised to 2 teaspoons (800 units) and to 3 teaspoons during the second month (1200 units).

Vitamin G (B<sub>2</sub>) or Riboflavin (Sherman and Langford, 1938)

Sherman and Langford grant that at present estimates of riboflavin needs for human subjects rest on no established test methods. They merely cite Rose and Stiebeling figures which are in close agreement. Stiebeling's are given in table 13. Rose suggests 400 units per day for children up to 10 years or 20 units per 100 calories if more than 2000 calories are consumed; for adults 20 units per 100 calories.

In Sebrell and Butler's cases of ariboflavinosis cures were produced with dosages of 0.025 to 0.05 mgm. daily or 8 to 16 units per day per kilo of body weight. This would mean 560 to 1120 Sherman-Bourquin units per day for a 70 kilo adult or 1400 to 2800 micrograms riboflavin daily.

# Other Vitamin Requirements of Normal Individuals

Of requirements for the other vitamins to prevent appearance of deficiency symptoms we have very little knowledge at present. Elvehjem suggests 25 mgm. nicotinic acid daily for pellagra prevention. Such information awaits development of diagnostic signs of deficiency, the availability of pure products for use in studies and better data on distribution in common foodstuffs.

# Requirements Under Pathologic Conditions

The problem of the physician in vitamin therapy is quite different from that of the dietician in planning reasonably adequate vitamin menus.

His first problem is accurate diagnosis of the causes of the deficiency which may be dietary inadequacy and may be faulty absorption and utilization. For that purpose, even more than the dietician, he needs the development of accurate clinical tests for measuring individual deficiencies and for following the results of this therapy. In general, too, he will make use of far higher dosage than is required for maintenance of normality for to restore depleted supplies and initiate recovery it is generally true that much larger amounts of vitamin are necessary than those given in the estimates cited in the preceding pages.

There is also accumulated evidence that since vitamin deficiencies are rarely specific but often a result of a combination of deficiencies he has the problem of synergistic relation between the vitamins, the influence of one upon another in the treatment of disease. The accompaniment in pellagra of peripheral neuritis and ariboflavinosis has already shown that a combination in such cases of B<sub>1</sub> and riboflavin with nicotinic acid is indicated for the deficiency is multiple.

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### CHAPTER V

# THE NATURE AND FUNCTIONS OF VITAMIN A

#### FORMS OF VITAMIN A

Vitamin A exists in nature in at least three forms. In plants it is in the provitamin form of carotene or cryptoxanthin. When these pigments are assimilated they are converted in the animal into nearly colorless compounds, active vitamins A. Two active forms of vitamin A have been found; designated respectively as vitamins  $A_1$  and  $A_2$ .  $A_1$  is found in the livers of salt water fish and  $A_2$  in the livers of fresh water fish. After ingestion provitamin A or the active forms may be found in blood and tissues in the free alcohol form or as esters.

It is definitely known that both carotene and active vitamin A circulate in the human blood. It has also been shown that there is an enzyme in the liver which is capable of converting provitamins (carotenes and cryptoxanthin) into the active form. Whether this can be accomplished in other tissues of the body has not yet been satisfactorily demonstrated. There is evidence to indicate that the thyroid gland plays a rôle in this conversion. Goats as a rule convert all the carotene to colorless vitamin A and produce a white milk. Thyroidectomized goats produce a yellow milk.

The chemical nature of these forms of vitamin A has already been discussed. Man's supply of these factors comes mainly from plants in which, as stated above, the vitamin is in the provitamin form. Active vitamin A is available in the butterfat of milk, the yolk of eggs and to a lesser extent in certain animal fats. It is abundant in fish liver oils. Neither the vitamin nor provitamin is water-soluble. Both are soluble in fats.

### FUNCTIONS OF VITAMIN A

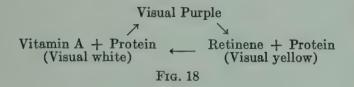
It is a label requirement of the Food and Drug Administration that when the potency of vitamin A is displayed on a commercial label, proof of such potency shall have been established by the rat growth test prescribed by the U. S. Pharmacopeia. The test demonstrates vitamin A potency by growth restoration in rats previously depleted of the vitamin but supplied with adequate amounts of all other known vitamins and essential nutrients. Growth stimulation then, is one property of vitamin A but it is a non-specific function since deprivation of any vitamin or other factor essential to an animals' nutrition will, of course, arrest growth and cause weight loss.

One of the earliest, if not the earliest manifestation of vitamin A deficiency in the human being is night blindness or hemeralopia. This effect is explained by the discovery that vitamin A is an essential constituent of the pigments which the retina of the eye uses to register visual stimuli. These pigments are found in the rods and cones of the retina. The rod pigment is called visual purple or rhodopsin; the cone pigment is visual violet. Both contain vitamin A attached to a protein.

Modern theories of vision indicate that objects become visible to us in much the way the camera produces photographs. The eye, like the camera, possesses a lens which focuses the image on the retina which corresponds to the film or plate in the camera. We know that in the photographic plate or film the image is made visible by a chemical change which takes place in the silver emulsion due to the impinging of the light rays on it. In the human retina the substances that correspond to the silver salts of the photographic plate are pigments found in the rods and cones. These pigments, as stated above, are the visual purple (rhodopsin) in the rods, and the visual violet in the cones.

When light rays impinge on visual purple it is bleached to a yellow substance called retinene. It is this bleaching of the visual purple which produces the stimulus to the optic nerve which we translate into vision. "Nature" states that in the bleaching of this visual purple there is probably an increase in the alkali groups available for ionization. At any rate, once bleached this pigment cannot again respond to light until it has been changed back to its original condition and it is in this regeneration of the retinene to visual purple that vitamin A functions.

Prior to 1936, various observers had reported finding vitamin A or carotene in the retinas in the eyes of different kinds of animals. Wald was first to show the significance of this



material in the eye and its relation to rod vision and Hecht showed its relation to visual violet regeneration in the cones. The relation between visual violet and vitamin A was suggested by Edmund and Clemmenson and confirmed by Hecht and coworkers.

In brief, the effect of an inadequate supply of vitamin A is to slow down the rate or regeneration of these pigments and hence to delay the speed with which an eye that has been exposed to light can recover sharpness of vision. The steps in the use of vitamin A for the regeneration of retinene are pictured in figure 18.

Retinene is a yellow pigment which has been shown chemically to be related to the group of carotenoids of which carotene is an example. Visual purple appears to be a protein combined with vitamin A. By adding vitamin A to retinene and protein visual purple is regenerated. In ordinary re-

generation part of the vitamin A for this reaction is supplied by the retinene. This reversible reaction between visual purple and retinene is not one hundred per cent efficient. We now know that the eye must receive from the blood a constant supply of fresh vitamin A to supplement that derived from the retinene and that if this constant supply is not maintained the rate of regeneration of visual purple is delayed. The result is that it becomes more difficult for the eye to adapt itself after exposure, to the reception of new images. It also makes it less efficient under conditions of dim lighting and hemeralopia is defined as failure to see clearly in dim light.

Lack of adequate vitamin A in visual pigment regeneration may also be a factor in determining the length of period between loss of vision due to the glare of approaching automobile headlights and ability to see sides of the road.

Various instruments have been devised to measure the extent of hemeralopia in the human eye and such instruments (properly used) give very definite proof of lack of adequate supply to the retinal layers. When such findings are interpreted as indicating lack of adequacy in the diet it should be borne in mind that such inadequacy might be due to failure to absorb ingested vitamin A or blockage of another sort between the digestive tract and the point of delivery. In general the hemeralopia, as determined by such instruments, should not be considered proof of inadequate dietary vitamin A unless treatment with vitamin A is shown to produce a definite relief and cure of the condition. With such check, measurements of this sort can be satisfactorily used to determine vitamin A requirements in a normal individual and have been so used in estimating vitamin A dietary needs.

Vitamin A fulfills a second highly specific function in the epithelial tissues of the body which is recognized by the structural changes associated with vitamin deficiency. This is metaplasia or a change in the character of various epithelia. The process presumably is a form of degeneration and suggests

that the vitamin is necessary for the full evolution of certain highly specialized epithelial structures; that when vitamin is withheld these are replaced by a less exacting kind of cell. Whether all instances of epithelial metaplasia are immediately due to vitamin A deficiency is unknown but the distribution of the lesions in vitamin A deficiency and their manner of evolution constitute a characteristic of this disorder alone. These lesions are discussed later on. It is important to point out here that alterations in function also result. duct of a gland which has become metaplastic and obstructed will completely suppress the function of that part. This may happen for example in the salivary glands and the pancreas. And very probably metaplasia follows changes of function and represents an advanced stage of the morbid process. In the trachea, for example, the epithelium first loses its ciliary function and later becomes metaplastic. Functional changes in epithelia may be much more important a part of vitamin A deficiency than is now recognized. Herrin's recent announcement that a decreased urea clearance of from 23 to 77 per cent occurs in deficient rats and that neither the kidneys nor the urine are abnormal in appearance could be interpreted as a purely functional effect on the renal epithelium.

While metaplasia does not occur in the intestinal epithelium Manville states that there is an absolute decrease in the number of goblet cells in the intestinal mucosa. And in the discussion in Chapter XXIII of the effect of vitamin A deficiency on resistance to infection work is quoted which indicates that the secretory ability of the cells of the intestinal tract is altered.

The requirement of epithelium for vitamin A may be demonstrated in vitro. Depletion of culture media of vitamin A by absorption with charcoal will stunt the growth of explanted epithelial cells and growth may be restored by the addition of vitamin as extensive experiments of our own have shown. Presumably the effects claimed by Lohr and others of vitamin

A rich ointments in the healing of skin wounds is due to the direct effect of vitamin A. Whether vitamin A plays a part in cellular oxidation has not been demonstrated.

## THE ABSORPTION OF VITAMIN A

It has been demonstrated by various investigators that both vitamin A and carotene are absorbable through the skin. Mackie and Eddy have reported six cases of ulcerative colitis in which the patient failed to respond by increase in blood level to very large doses of vitamin A by mouth and responded quite promptly to inunctions of cod liver oil on the chest. Eddy and Howell showed that both vitamin A ester and carotene were rapidly absorbed by intact rat skin. Eller and Wolfe have recently reviewed data on skin absorption of both vitamins A and D.

Incidentally the data on the relative absorbability of carotene and active vitamin A from the digestive tract are also somewhat conflicting. Van Eekelen and Pannevis reported that when carotene was ingested in the form of spinach or carrots 94 to 99 per cent was eliminated in the feces unabsorbed, but when an equivalent amount was dissolved in oil, only 41 per cent was excreted. In direct contradiction to this, Bradfield and Smith found that in feeding dogs on a level of 20 International units per 100 grams of body weight the carotene of carrots was as well utilized as carotene in oil. We need further data on the mechanism of both vitamin A and carotene absorption.

Clausen and McCoord have studied extensively the effect of vitamin A feeding on the blood content of infants between the ages of birth and two years. They report that the carotene content of the blood is lowest at birth and reverses later but that in general, fed carotene is more slowly absorbed than active vitamin A. Dutch workers report that cryptoxanthin is not so rapidly utilized as alpha or beta carotene.

Clausen suggests that the slower absorption of carotene

may be due to the fact that it cannot form esters. If conditions favorable to the absorption of fats in general are essential to the absorption of vitamin A, it will be obvious that other conditions unfavorable to fat absorption will directly effect vitamin A absorption. For example, diarrhea, pancreatic dysfunction with inadequate supply of lipases as well as disturbances in liver function and bile formation may all be concerned in the control of vitamin A.

Ethyl alcohol, whether administered parenterally or orally mobilizes vitamin A in dogs. Clausen and associates report that in some animals the blood concentration was doubled. A lesser effect had been noted in rabbits following ether anaesthesia and after severe bleeding.

Dutcher and coworkers found that when mineral oil was fed at the same time as vitamin A source, the feeding of the A as carotene resulted in greater loss than when active vitamin A was fed and explained the difference on the lesser solubility of the active A in the mineral oil.

As stated above, we do not know much as yet of the mechanism of conversion of carotene to active A. An enzyme capable of such conversion and called carotenase, has been isolated from the liver but in-vitro experiments have proved disappointing. Drummond showed that a colloidal, aqueous suspension of carotene injected into the portal or systemic circulation was rapidly removed by the reticulo-endothelial system. Sections of liver after injection showed granules in the Kupffer cells which slowly disappeared as the A content of the liver increased.

Phosphorous poisoning does not destroy the Kupffer cells or affect liver retention of A but it does destroy the parenchymatous cells and reduces the conversion of carotene to A; leading to the belief that the parenchymal cells may play a rôle in carotene conversion.

With present methods of measuring the blood content of vitamin A and of carotene, we can determine the effect of

kinds of vitamin A ingested upon the blood level of these forms. In the study of visual adaptation there seems general indication that if the individual is in normal health he will obtain his needs whether the source be mainly carotene or active vitamin A but it should be evident from this brief discussion that generalizations as to the relative value of vitamin sources are dubious until we know much more of the methods of conversion of carotene and of the factors that control the absorption, storage and utilization of these substances.

## STABILITY OF VITAMIN A AND CAROTENE

A matter of greater immediate concern than the form of vitamin A ingested is the matter of stability of the vitamin. All the vitamins can be totally inactivated by certain chemical measures and vitamin A or carotene when heated in the presence of oxygen or an oxidizing agent rapidly loses its physiological activity. A fundamental requirement for maintaining potency of this vitamin is therefore the avoidance of oxidative processes. Hauge and Aitkenhead also believe that carotene can be inactivated by enzymatic processes. Russell showed that alfalfa, quickly dried artificially, lost far less carotene potency than alfalfa allowed to dry in the field. The quick drying process rapidly inactivates the enzymes. We have also seen that hydrogenation destroys activity and if our theory is correct this involves, like oxidation, the saturation of the double bonds in the polyene alcohol chain.

Various methods are now employed by drug houses to insure the label potencies stated on their packages. Some employ vacuum methods of sealing their bottled fish liver oils, some use antioxidants such as wheat germ oil. The American Medical Association Council of Pharmacy does not sanction the use of hydroquinone for this purpose but harmless antioxidants may be used. Most drug and food manufacturers incorporate a certain percentage above the label claim against possible deterioration and to insure finding of all the potency

claimed. If one purchases medicinal or diet supplement capsules of vitamin preparations from reliable firms today one need not greatly fear lack of claimed potency as stated on the lable and the same holds true for fortified foods.

In the home, cooking operations as a rule do not seriously menace vitamin A activity. Kohman and Eddy found little destruction of vitamin A by the commercial canning process; Fellers showed little loss of A in freezing foods; and Morgan showed that the sulfuring of fruits conserved rather than accelerated the destruction of vitamin A. Avoidance of fermentation and of oxidation is desirable to maintain vitamin A potency at full strength and precautions in this direction will insure adequacy of the factor if the original source contains the active vitamin.

#### TOXICITY OF VITAMIN A

Vedder and Rosenberg fed jewfish liver oils containing approximately 600,000 International units of vitamin A per gram to rats in daily dosages of 25,000 to 100,000 units daily. They saw no evidence of injury and stated:

If vitamin A is ever toxic it is in excess of 100,000 I.U. daily for 50 gram rats.

The author has observed daily dosages of vitamin A in amounts as high as 100,000 I.U. to patients at Roosevelt Hospital in New York, using percomorph oil, with no signs of toxicity. On the other hand, reports of cachexia, loss of weight, fragility of bones, and skin changes; also inflammation of the eyes have been reported by Weslaw, Wronski, and the Wroblewskis with the use of a vitamin A preparation called "Vogan." They found injection of the product more active in impairment of tissue than when the vitamin was supplied by oral administration.

Experience with cod liver oil inclines one to question whether their results were due to high A intake or to other factors in the preparations used. In 1926, Agduhr called attention to serious morphological and functional changes associated with high doses of cod liver oil. These results were duplicated to a certain extent by Cornell experimenters but the opinion of students of the problem today is that some factor in the oil other than vitamin A was the responsible cause. It is also true that the effect of vitamin D in "Vogan" preparation was not eliminated.

It may be stated at present writing that there is no significant evidence of harm from high dosage of vitamin A in man.

# OTHER MANIFESTATIONS RELATED TO VITAMIN A NATURE AND FUNCTION

One reason for the seasonal sales of cod liver oil and other vitamin A preparations has been the stress put on the value of vitamin A for the prevention of colds. This is unfortunate for two reasons: First, the vitamin A need exists through the year, it is not seasonal; and second, the claim is not fully warranted.

Varying results of vitamin A treatment for colds have been obtained by the following investigators; Shibley and Spies, Beard and Cameron. Most investigators are agreed today that vitamin A dosage shows little value in reducing the incidence of colds but that those so infected tend to recover more quickly if they have built up reserves of this vitamin and that their colds are of shorter duration.

The Council of Pharmacy of the American Medical Association states its viewpoint in regard to claims for vitamin A as follows:

Present indications are that vitamin A is an aid toward establishing of resistance of the body to infections in general only when there has been a decrease in body reserves of the vitamin and the ingestion of vitamin A is inadequate. It has not been shown to be specific in the prevention of colds, influenza, and such infections, nor has it been demonstrated that ingestion of vitamin A far in excess of that necessary for normal body function, and readily obtained from a properly selected diet, is an aid in preventing various types of infections.

In the matter of infection then and expecially infections of the respiratory tract it would seem not unreasonable to urge adequacy of vitamin A in the diet as one means of maintaining normal resistance but not as a cure or a specific preventive of any single type of respiratory infection.

## EXPRESSION OF VITAMIN A POTENCY

With the identification of vitamin A as a definite chemical substance it is now possible to express the vitamin A content of a source in amount of vitamin present; and since betacarotene to date appears to be the form of the vitamin of highest potency the League of Nations Vitamin Committee defines a unit (International or U.S.P.) as the amount of vitamin source that produces the physiological effect 0.0006 milligrams of pure beta-carotene. It is difficult to match the effect of a source against beta-carotene itself so in Europe and in America it is customary to use reference standards for that purpose. The International reference standard is a solution of pure beta-carotene in inert oil such as cotton seed, peanut, or refined olive oil in such amount that 1 gram of this oil contains 0.003 mgm. of beta-carotene. In the United States the U.S. Pharmacopeia Committee has provided as a reference standard a special cod liver oil that has been proven to contain 3000 International units of vitamin A per gram.

In the Appendix of this text the reader will find the approximate vitamin A content in International Units per 100 grams of common foods. When this amount is multiplied by 0.0006 it gives the beta-carotene content or its equivalent in active vitamin A. Convenience has established the practice of expressing carotene values in gammas or micrograms. An international unit is the equivalent of 0.6 Y.

## VITAMIN A REQUIREMENT

The quantity of vitamin A necessary to maintain normality, the amounts necessary for optimum nutrition, and the amounts

necessary for the cure of conditions resulting from vitamin A deficiency are necessarily all different. In the interests of uniform labelling the Foods and Drugs Administration in Washington has suggested the following as minimum daily requirements (Federal Register, March 28, 1940):

|   | I. U. |
|---|-------|
| For infants not more than 1 year old                          | 1500  |
| For children older than 1 year but less than 12 years of age. | 2500  |
| For all persons over 12 years of age                          | 3000  |

By minimum requirement they presumably mean the least amount necessary to prevent visible symptoms of vitamin A deficiency.

These figures have been arrived at by review of various studies. (See Chapter IV for general discussion of the problem of vitamin requirement.) We may suggest, however, that until we have better diagnostic tests for A deficiency and better methods for measuring the effect of vitamin therapy all figures on requirement must be considered as estimates of needs rather than proven requirements.

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## CHAPTER VI

## AVITAMINOSIS A

Vitamin A deficiency results in three cardinal signs, xerophthalmia, dermatosis and night blindness.

Hemeralopia has been recognized since Hippocrates. If we turn to a description of this condition written shortly before the discovery of vitamins, we find that "it has been oftenest observed as an acute epidemic infection attacking large numbers of people living under nearly identical abnormal condition." "Older writers have said it especially affects persons with pigmented eyes." (We now know that pigmentation of the eyes is another expression of A deficiency.) "Impaired nutrition and strong sunlight have almost always been associated with its occurrence." (The effect of light on visual purple has been explained.) "Dryness of the scleral conjunctiva with formation of scaly patches—conjunctival hyperemia with lachrymation and photophobia—are frequently associated."

In Hippocrates' time, too, they had discovered a treatment whose basis is understandable only since the discovery of vitamin A. To them it was purely empirical. An ointment was made from the juices exuding from roasting liver and applied to the eyes, or they were steamed with vapor from the water in which liver was cooked; to which was added the recommendation to eat the liver. Cod liver oil was also highly commended.

It may be seen from the foregoing that long before the discovery of vitamin A physicians were familiar with the major features of its deficiency effect and had empirically developed some sound measures of treatment.

# RELATION OF VARIOUS MANIFESTATIONS OF VITAMIN A DEFICIENCY BUT RECENTLY RECOGNIZED

The earliest lesion attributed to vitamin A deficiency was xerophthalmia which occurs in rats as well as man. This discovery explained the early empiric treatment of cases of xerophthalmia and the clinical observations of associated night blindness were promptly ascribed to the action of the vitamin as well. The identification of characteristic skin lesions came relatively late and was only established by the studies of Frazier and Hu. This no doubt was due to the irregularity with which skin lesions occur in human cases and their absence in the usual experimental animal, the rat.

#### MODERN OUTBREAKS OF THE DISEASE

Less interest in prevention seems to have existed in more modern times and the disease has persisted in many places, and has often occurred in major epidemics. Russia, Brazil, Denmark, and the Mediterranean lands, have all had epidemics of hemeralopia and xerophthalmia, and cases are still common in China and India.

In Tientsin six per cent of the patients attending an eye clinic in 1929–1930 had conjunctival xerosis. Nicholls gives the incidence of the disease in Indian institutions he had inspected as follows:

| p                                 | er cent |
|-----------------------------------|---------|
| Charity boarding schools          | 83      |
| Poor vernacular schools           | 29      |
| Upper class schools               | 3       |
| Mental asylums                    | 44      |
| Mental asylums (on European diet) | 2       |

The disease is also common in Yucatan and in Labrador. Pillat says that the poorer Chinese are almost permanently on the border line of vitamin A deficiency.

Many epidemics of night-blindness occurred during the first World War. Braunschweig's report in 1915 was the earliest recognition that the condition was prevalent. Birch-Hirschfeld studied it extensively on the Eastern Front. Among French physicians it was reported by Wecker in 1916. Of 4000 soldiers referred to his clinic 409 were found to suffer from hemeralopia. Tricoire was one of the first to associate its occurrence with vitamin A (but recently discovered). In one army unit Tricoire found 300 cases, 6 being complicated by scurvy. The symptoms were frequently used as an excuse from duty according to Codvelle. Nicolau, who observed the Roumanian campaign and the same epidemic of scurvy which was studied by Aschoff and Koch, reported an outbreak of skin lesions resembling acne or syphilodermide. These he believed due to scurvy but the description leaves no doubt that they were an expression of vitamin A deficiency. Nicolau found that one out of every five cases of scurvy also had these skin lesions. The only previous record of them, according to Nicolau, was made by de Larrey, surgeon to the Armies of Napoleon.

Nicolau did not associate the disease with the epidemic of hemeralopia which occurred in Roumania at the same time and which principally affected children. Dr. Gideon Wells diverted cod liver oil from Archangel and saved the sight of many victims (Plimmer).

## THE PATHOLOGIC ANATOMY OF VITAMIN A DEFICIENCY

The pathologic anatomy of vitamin A deficiency is essentially the same in experimental animals and in man. However, the experimental evidence is more complete and orderly, and will be considered first.

# Epithelial Metaplasia

The specific anatomic effect in the rat, guinea pig, monkey, chicken and other animals, is the loss of ability to maintain various specialized epithelial surfaces. The result is the replacement of such surfaces by less specialized cells, a change

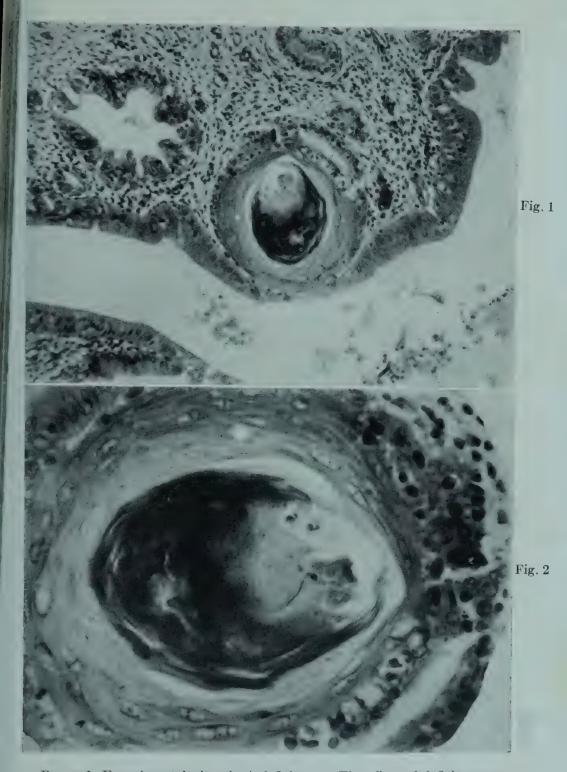


PLATE I. Experimental vitamin A deficiency. The effect of deficiency on the endometrium of a rat. Figure 1 shows the endometrial gland in the center of the photograph to have undergone metaplastic involution. The gland is filled with keratinized epithelium. Notice that the lesion is purely focal. Figure 2 shows the same gland more highly magnified.

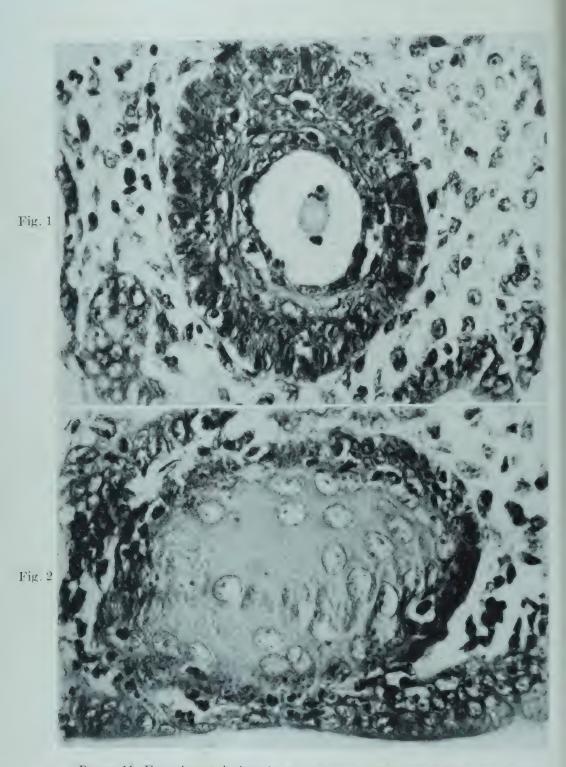


PLATE II. Experimental vitamin A deficiency. Endometrial lesions in the rat. In both figures the deeper cells may be seen to have been very active. Mitotic figures are numerous. The excess cells have piled up within the glands. In figure 1 the epithelium is stratified.

The lesions commence simultaneously in various foci. In the rat, a general order of development was pointed out by Wolbach and Howe: first the respiratory mucosa, then, in order, the salivary glands, eyes, glands of the gastro-intestinal tract, parocular glands, and pancreas. However, exceptions to this rule occur in the rat and among various species. Lesions of the eyes were not found in the monkey—Tilden and Miller—or guinea pig—Wolbach and Howe.

The complete picture of vitamin A deficiency in the rat and guinea pig has been drawn by Wolbach and Howe. By the time the rat is retarded in growth, assumes a hunched position, and shows encrusted eyelids, and a rough coat, keratinized epithelium may be found in the nares, larynx, trachea, bronchi, in the submaxillary, parotid and accessory salivary glands, in the urinary bladder, ureters and kidney pelves, the uterus, oviducts, prostate, epididimis and seminal vesicles, the conjunctiva, cornea, lacrimal glands and the ducts of the Meibomian glands, and in the thymus, where the Hassall corpuscles are magnified and often transformed into cystic spaces filled with keratinized epithelium. Similar changes occur simultaneously in the enamel organ leading to extensive changes in the teeth.

In all of these sites but the enamel organ, the process is identical; and in that organ substantially so.

In the early stages of the deficiency, nests of darkly stained germinal epithelium may be seen to undergo rapid growth. As it grows, the secretory or duct epithelium overlying degenerates and sloughs off, and the foci grow to form islands of stratified squamous epithelium. These first extend laterally to undermine the adjacent surfaces and eventually, if the dietary defect is sufficiently prolonged and severe, extend to replace the entire surface of the affected organ. With lesser deficiency, alternating areas of normal and replacement epithelium occur side by side, and the former remains normal in appearance.

The process begins distant from other stratified epithelium and quite independent of it. According to Seifried the most important vitamin A deficiency in domestic animals occurs in chickens where it causes serious economic losses and is usually confused with common infectious diseases, chicken pox, coryza contagiosa and infectious bronchitis. Symptoms appear 40 to 60 days after inauguration of deficient diet and death occurs a month later. The nasal passages and sinuses are filled with masses of clear mucoid material which later becomes opaque and caseous. If it is removed the membranes beneath are found to be dry and opaque. In the larynx and trachea thick patches of caseous material collect in the mucous glands and crypts and the mucosa is likewise metaplastic and dry. Seifried closely followed the histological sequences. These start with atrophy, loss of cilia, shrunken nuclei and loosening of masses of epithelium. New cells form beneath, often in syncytial masses and grow to form areas of flattened squamous epithelium. Keratohyalin granules may be found in the cells. Balloon giant cells occur and mucous secretion, of course, ceases. Particular attention was paid to phenomena which occur in the nuclei of degenerating cells and which often end in the extrusion of nucleoli which then resemble Guarnieri bodies. The lesions in the mouth resemble those of fowl pox. The submaxillary glands are affected early in the disease and, with occlusion of the ducts, often become distended and cystic.

Some disagreement exists as to whether the metaplasia should be regarded as a true or replacement metaplasia. Our

opinion is that the latter is the more probable explanation because of the evidence which shows epithelial deterioration preceding metaplasia. This consists of the opinion that in the trachea the ciliated epithelium shows loss of function before structural changes occur; the evidence of Pillat that in the corneal epithelium serious damage occurs in the mitochondrial apparatus—which contains much lipoid—and in the Golgi structures before frank lesions appear; and the discovery that the purine content of tissues are reduced during deficiency. The purines are required in the formation of cell nuclei. To these reasons might be added the general observation that it is more harmonious with what is known of other vitamin deficiencies, to consider that a material essential to specialized cells is lacking in vitamin A starvation.

The primary consequence is the loss of function of the affected surface. In the case of the trachea, the loss of cilia precludes proper cleansing of that part, and in the conjunctiva the loss of mucus secreting cells has a similar adverse influence. Similar effects must occur elsewhere although they are less obvious. Two other major sequelae have been repeatedly emphasized.

The most striking is the blockage of gland ducts leading to stasis of secretions and, with the interruption in the intact surfaces, to infection. The frequency of infection in organs diseased as a result of vitamin A deficiency seems to have varied in different experiments, probably in part due to the degree of cleanliness of the cages and animals, and led earlier experimenters to consider that infection invariably occurred and predisposed to the metaplasia.

This is not the case, as Wolbach and Howe and others have shown. Proof is also afforded by the study of the lesions of the enamel organ, the uterus and oviducts where infection rarely occurs.

The associated abscesses and metaplastic changes have always been most common about the mouth and its glands

where exposure to infection is obviously easy and, indeed, the rule. Nevertheless, complicating infection is very common. Indeed infectious lesions account for the death of many experimental animals if the closest attention is not given to prevent them. Orten, Burn and Smith report a mortality of 54 per cent in their experiment, described elsewhere, in which deficiency was maintained for exceedingly long periods. The causes of death were tracheal obstruction (mucous plug) otitis media and sinusitis.

Mellanby reported that examination of 92 rats on an A deficient diet showed that 44 per cent had infection of the genitourinary tract including pyelonephritis, cystitis, renal and bladder calculi, hydronephrosis, dilated ureters, and distended bladders; 20 per cent had otitis media; 20 per cent had acute inflammation of small or large intestine—acute enteritis involving duodenum and jejunum and hemorrhage at the pyloric end of the stomach; 9 per cent had lung infections; 38 per cent had xerophthalmia; 72 per cent had abscesses in the floor of the mouth.

The other significant consequence of the epithelial changes lies in the interference with the functions of the glands and organs involved. Wolbach considers these effects as sufficient to explain the anemia and cessation of growth. The lesions in the uterus and germinal cells produce sterility. A completely obstructed kidney is occasionally seen. Urinary calculi are common. This will be discussed later.

## Tooth Changes

The tooth lesions were recognized later than the epithelial changes. They are equally striking and characteristic. Wolbach and Howe consider they are the most important dental effect of any of the deficiencies.

The deficiency expresses itself in atrophy and metaplasia of the enamel organ. Enameloblasts become replaced by stratified squamous epithelium. As a result, there is loss of

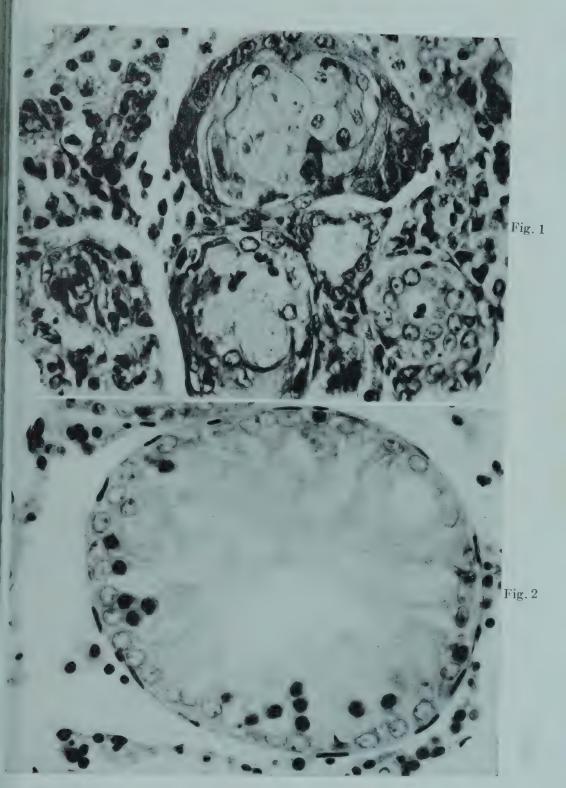


PLATE III. Fig. 1. Metaplasia in a small pancreatic duct as a consequence of vitamin A deficiency. Lesions of this kind are very common in the pancreas in experimental vitamin A deficiency. Fig. 2. A seminiferous tubule showing the effects of vitamin A deficiency in the rat. Spermatogonia and spermatocytes alone remain. The latter are limited to two small clusters of cells. The lesion is typical of the late effects of vitamin A deficiency.

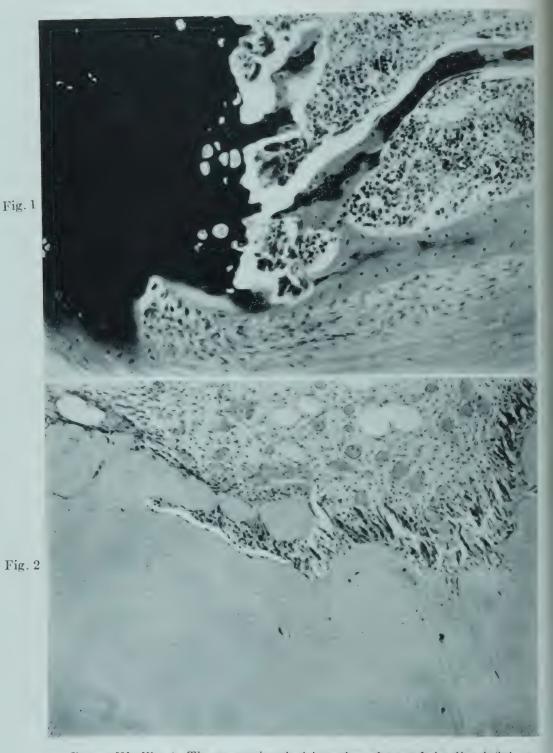


PLATE IV. Fig. 1. The costo-chondral junction of a rat fed a diet deficient in vitamin A. The photograph shows cessation of cartilage growth as well as atrophy of osteoblasts. These changes result in failure to grow. What bone is present is dense and substantial. Fig. 2. A lower incisor from the same animal. The photograph shows the buccal surface of the pulp. Atrophy of the odontoblasts was even more pronounced on the lingual surface. The denticle formation (center of photograph) is regularly seen in protracted cases of vitamin A deficiency. Notice that the dentin is dense although irregular.

enamel and exposure of dentin which gives the teeth a chalky appearance. Simultaneously, the odontoblasts within the tooth atrophy and an irregular line of thin odontoblasts with irregular islands of denticles form. This occurs last in the labial surfaces so that the tooth has a thicker labial than lingual wall. Schour, Smith and Hoffman measured these differences by injecting a dye (alizarine Red S) which stained the proximal margin of the dentin. At various intervals thereafter teeth were examined and the accretions of dentin could be readily measured. Whereas 16  $\mu$  formed normally teeth of deficient animals showed as much as 19  $\mu$  on the labial surfaces and as little as 6  $\mu$  on the lingual surfaces of the incisors.

Additional information has also been contributed by Orten, Burn and Smith who managed to prolong deficiency by feeding small vitamin A supplements adjusted to each particular animal. Thus an incipient vitamin A deficiency was maintained for periods up to 1 year. Growth was retarded but the growth curve had a normal configuration. The most striking and consistent finding was in the incisors which became opaque, distorted and twisted. Ridges appeared on the surfaces and in some cases the teeth fell out. Large masses developed in the maxillae. Odontomata and supernumerary incisors were occasionally found. Some of the former became large enough to erode the gingiva. They were formed of cells resembling odontoblasts and tooth buds could be found. The enamel organ was atrophic and metaplastic. The molars were not affected.

## Nervous System

Very contradictory views are held on the effect of vitamin A deficiency on the nervous system. Edw. Mellanby has consistently championed the view that not only are the nervous lesions a characteristic result of deficiency but that they are the fundamental disturbance, that the skin and eye changes

are the consequence of nerve changes. He has reported lesions in the fifth nerve in rabbits, dogs and rats. One dog became completely blind after a prolonged subsistence on A deficient food. Zimmerman's studies have shown lesions of the nerves in the sensory and crossed pyramidal tracts. Spasticity and clumsy coördination were observed. Rats became paralyzed in some cases within 40 days. Predominantly motor disturbances were described by Seifried including ataxia, incoördination and convulsions. Lesions were found in the spinal cord and peripheral nerves.

More recently Irving and Richards have reported extensive rat experiments which seem to be the most definite demonstration of the actual association of the deficiency and nervous lesions. Search was made for the symptoms described by Mellanby by using a tilting platform which exaggerated signs of lameness and incoördination. However even this failed to reveal disturbances in most of their animals. Histological studies, made with polarized light, gave rather uniform results. Lesions were found in the funiculus praedorsalis in 108 of 141 animals. The absence of lesions in the rest was believed due to the shorter period of test. The lesion occurred at the level of the pyramidal decussation but in many animals the spino-cerebellar tract and column of Burdach were also affected. None of the animals on the same diet which received supplements of carotene or vitamin A showed such lesions.

There has been a general tendency to consider other dietary or constitutional factors responsible for the lesions found in vitamin A deficient rats. However Aberle reported that they could not be prevented by considerable amounts of yeast. A further objection, that the technical methods used are undependable (the Marchi technique) has been met by Setterfield and Sutton by using polarized light. Myelin degeneration was found in the femoral and sciatic nerves in rats. Reiser partitioned the lipids in the nerves from these experiments

and found the saturated, conjugated lipids diminished, adding chemical evidence of an effect.

Lesions have not been found by Eveleth and Biester, Duncan and others. Wolbach wrote: "There is no substantial evidence that degeneration of myelin sheaths is a specific consequence of vitamin A deficiency. That it does occur in some strains of laboratory animals cannot be denied." That the histological changes in epithelium are due to nervous lesions seems very remote. The promptness with which metaplasia may be reversed after prolonged deficiency when, judging by the nervous changes reported the lesions there at best would be slowly reversible, seems strong evidence to the contrary. Furthermore there are no studies of the effect of nervous disturbances on epithelial surfaces in any way related to metaplasia. King failed to duplicate the lesions in the teeth by resection of the inferior dental nerve.

The discrepancies between various studies of the effect of vitamin A deficiency on the nervous system may in part be explained by a recent report of Wolbach and Bessey. They found that rats placed on a deficient diet when very young, the 21st day of life, showed ataxia and paralyses a month later. Degenerative lesions were present in the peripheral nerves, various spinal tracts and the cerebellar peduncles. age distribution was due to a mechanical effect. The nervous system continued to grow during the period of deficiency, and may possibly have grown at a greater than normal rate, while the bones of the spine, in common with the other parts of the skeleton, ceased growing. The central nervous system therefore outgrew its bony vault as evidenced by herniation of the nerve roots into the foramina and pockets in the bodies of the vertebrae. The nerves lay twisted and even coiled within these spaces. Herniation of the brain tissue into the venous sinuses was also seen and various distortions of the base of the skull which were attributed to pressure.

Reference should be made to the lesions of the rat retina

described by Johnson. An absence of droplets believed to be associated with visual purple occurs during deficiency and abnormalities in the staining reactions of the cells of the periphery of the retina occur in even moderate degrees of deficiency. In severe avitaminosis more extensive, progressive degeneration is found. Degeneration affects the parts of the retina in the following order: rod segments, commencing peripherally; external limiting membrane; outer nuclear layer, epithelial pigment layer; outer molecular layer and the inner nuclear layer. The most severe injury is present in the fundus of the retina.

# Urinary System

Higgins, McCarrison, Van Leersum and Steiner, Zuger and Kramer, to mention but some, have all found renal calculi common in deficient animals. The calculi are usually composed of calcium and tend to disappear if the diet is corrected. The opinion held by most investigators is that these are simply concretions formed about desquamated epithelium, a consequence of metaplasia of the kidney pelvis, ureters and bladder. Most of the studies have been made in rats which are prone to spontaneous calculi but Steiner, Zuger and Kramer were also able to produce calculi in 9 of 35 guinea pigs fed a vitamin A deficient diet. Mineral intake and pH of the urine were controlled in these experiments. Wolbach, however, believes other factors than the vitamin are involved since calculi did not occur in his animals. Erspamer searched for gall stones in deficient guinea pigs. Amorphous masses were found but nothing resembling calculi such as occur in man. (Many of the renal calculi which have been described seem to have been but inferior imitations of those which occur in man.) No success has followed attempts to treat renal calculi in humans with vitamin A. However cases may well occur, but rarely, in which deficiency and kidney pelvis metaplasia may incite the deposition of a urinary stone. Metaplastic lesions are not rare in human material and if the character of the urine is suitable should predispose to stone formation. That this mechanism is responsible for other than exceptional cases seems unlikely.

# Lesions of the Skin

In the light of what has been learned of human skin behavior in vitamin A deficiency, it is worthy of mention that similar lesions have not been described in rats.

The changes observed by Wolbach and Howe were atrophy of hair follicles and sebaceous glands, but not distended, keratinized follicles.

Skin lesions in rats are commoner in animals more than four months old. They appear as scabby ears and tails, sores or abnormal growths on the nose, sore feet and ragged hair.

# Organs of Reproduction

Vaginal lesions lead to abnormal numbers of cornified epithelial cells in vaginal smears.

Evans and Bishop recommend examinations of vaginal smears as a dependable and early criterion of vitamin A deficiency. Success and failure with this method have been reported by others. The recent studies of Mason seem to explain the failures and to establish the test on substantial ground. Mason observed that the first effect of the deficient diet was to exaggerate and prolong the appearance of cornified cells during estrous. Later the cornified estral phase becomes more and more prolonged until the entire cycle is characterized by cornified vaginal cells. In other words, the estral cycle must be followed in the early stages of deficiency to reveal the first pathological changes.

Pregnancy exaggerates the inherent weakness of the genital organs when vitamin A is deficient. Intrauterine death and resorption occur when the deficiency is exaggerated.

The site of the lesions is unpredictable with here and there

along the horns of the uterus a diseased implantation site. The

histological changes are, however, very constant.

The maternal decidua becomes necrotic and infected, and the consequent interference with foetal nutrition leads to death. The damage is primarily to maternal cells in distinction to deficiency of vitamin E in which the first effect is on the foetal tissues. Vaginal hemorrhages with necrotic cellular debris, often with foul odor, announces the occurrence of fetal resorption.

By increasing the vitamin A intake somewhat, just sufficiently to keep animals on the border line of the earliest lesions of vitamin A deficiency, a variety of other abnormalities may be produced including prolonged gestation, difficult labor, sometimes with death of foetus or mother, retained and diseased placentae with areas of hemorrhage and leucocytic infiltration and a high mortality among the new born during the first five days of life. Difficult labor seems in part to be due to the cornified vagina which makes the expulsion of the foetus difficult; and to loss of tone of the abdominal muscles. Whether the latter is due to degeneration of skeletal muscle as described by Wolbach, with loss of striation, swelling and degeneration of fibers, is not determined.

It cannot be considered established that the effect of the deficiency operates directly on the decidual cells since degeneration of them has so far been seen only in association with infection. The degeneration may, therefore, be the result of infection.

Persistence of the epithelial cells often occurs between trophoblast and decidua and associated with necrosis and inflammation. Mason, to whom we owe these studies and description, considers that the deficiency has caused these epithelial cells to undergo degenerative changes which have prevented their digestion by the trophoblast.

The high mortality rate among the new-born may be due to poor mammary function as well as weakness of the young.

Mammary function is inconstantly present and may be due to morphological changes in the breast since these are character-listically inconstant. Presumably congenital defects, as well as weakness, may appear in the young. Hale has extensively studied this phenomenon in swine and found microphthalmia, hare lip, cleft palate and malposition of the kidneys. In a rat experiment recorded by Browman 6 cases of microcephaly were found among 782 young. However, Cannon, and others, have failed to produce congenital defects in rats. It may be, as Cannon suggested, that the level of depletion in Hale's experiments was lower than in his own, that swine mate, conceive and carry young on vitamin A intakes which cause sterility in rats.

There is much evidence that the effects of a previous deficiency persist throughout the life of an animal. In Browman's work, to which we have just referred, female rats were depleted and then fed ample amounts of carotene. Fertility was permanently reduced and birth mortality high despite the absence of lesions in the mothers and the young. Similarly Sherman and MacLeod found that female rats reared on a partially deficient diet were permanently stigmatized. They suffered a high mortality during young adult life (mainly due to respiratory diseases) and were unable to rear a second generation. Mutch and Richards have found that rats which have had xerophthalmia show corneal nebulae and myopia which are permanent.

## Other Lesions

Emaciation is regularly found in vitamin A deficiency as is atrophy of many organs, viz., the testes, thyroid, pituitary gland, as well as the salivary glands, liver and spleen. The changes in the testis will be discussed under the pathogenesis of vitamin E deficiency. They are not specific.

In addition, extensive changes are to be found in the bones. The lesions in the long bones are similar to those in the dental pulp, in that atrophy is the most conspicuous histologic feature. The cartilage ceases to differentiate, and bone grows slowly over the cartilage plate. Atrophy is also evident in the spongiosa which is defective, being formed of a few thin trabeculae, and, wherever seen, the osteoblasts are small and

atrophic.

The cartilage and bone itself are densely calcified, and evidently of a very substantial nature. Bone previously formed is well maintained. This characteristic alone is of considerable importance in the identification of the bone lesions in vitamin A deficiency, and contrasts greatly with the appearance of cortical bone in scurvy for example, where it is less opaque and more delicate, and where the osteoblasts are surrounded by thin, palely stained, material. Since growth ceases, it is not surprising that osteoid tissue is scanty or lacking.

While these lesions are not characteristic enough to afford definite means of differentiating vitamin A deficiency from certain other dietary defects, or starvation, they afford no reasonable basis for the confusion that has existed in the past.

The digestive tract is probably affected secondarily in that interference with the associated glands may well produce disturbed function. Richards believed it was primarily affected and found pits and erosions to be common throughout the bowel as well as hemorrhages and duodenitis and colitis. This has not been the experience of others. Hyperkeratosis and ulceration of the forestomach of rats has been observed by various writers since first described by Pappenheimer and Larrimore. They believed that ingested hair had much to do with the development of the lesion. More recently Andervont has shown that strain differences are important in the etiology of this lesion. The reports of Howes and Vivier and of Sharpless indicate that the lesion is due primarily to a deficiency other than vitamin A. Thus Howes and Vivier demonstrated that yeast supplements were preventive and Sharpless that a supplement of flavine, nicotinic acid, cystine and rice polishing concentrate was likewise preventive.

One consequence of the study of gastric hyperkeratosis has been a number of experiments designed to determine whether this lesion plays a part in the causation of gastric carcinoma. Positive results were claimed by Fujimaki but others have failed to duplicate his results. A comprehensive investigation has recently been described by Fridericia and associates from Fibiger's laboratory. Fridericia and his colleagues found papillomata more common in deficient rats but were unable to produce carcinoma. Even the papillomata appeared not wholly or directly due to vitamin A deficiency since they occurred in some of the control animals. It may be that the immediate mechanism in keratosis of the stomach is vitamin A deficiency, that other factors induce a local deficiency effect such as we have discussed elsewhere. The character of the lesions suggests this.

## Visualization of Vitamin A in Tissues

An extremely promising technique in studying the lesions caused by deficiency is afforded by fluorescent microscopy. Several articles by Popper will be found valuable. Popper demonstrated that the fluorescing droplets contain vitamin A and studied their distribution among cells and organs. The Kupffer cells of the liver contain vitamin A and the amount is increased during febrile disease. Inflammatory lesions altered the distribution of the vitamin. In the hepatic epithelium the amount varied but was reduced by exhausting disease, acute hepatitis and cirrhosis. Droplets are common in the adrenal cortex, in the corpus luteum and the Leydig cells of the testis. They appear in the kidney epithelium only in association with nephritis.

Goerner and Goerner have described instructive studies which also penetrate the cell in exploring the rôle of vitamin A. They were able to show that dibenzanthracene, a carcinogenic compound, caused decrease in the amount of hepatic vitamin A. The vitamin A of the mitochondria of the liver cells disappeared and large amounts of vitamin A administered paren-

terally failed to replace this supply. Tumor cells likewise lack mitochondrial vitamin A. These studies have the further significance of exposing a disturbance of vitamin A metabolism in individual cells rather than in organs or the animal as a whole.

## "HYPERVITAMINOSIS A"

A variety of lesions have been produced in young rats by feeding very large amounts of certain vitamin A concentrates. These are described as constituting "hypervitaminosis A." Among the lesions which have been most frequently described are trophic changes in the skin, bones and bone marrow. skin lesions appear first. The hair becomes coarse and dull and later, commencing at the nose and ear margins, falls out. Ring crusts form on the tail. Thick secretion and inflammatory changes appear in the conjunctiva and rhinitis is often associated. The skeletal lesions include atrophy of the long bones which become very thin and brittle. Spontaneous fractures are common. These lesions were first reported by Collazo and his associates and have since been found by a number of other investigators. Rather different lesions were described by Drigalski; nephrosis (which was the usual cause of death) and toxic degeneration of the striated muscles and of the testis. Drigalski also observed the nasal and eve lesions. General agreement exists of the effect on the blood. A hypochromic anemia occurs with signs suggesting retarded erythrocytic maturation and a granulocytopenia. In most of these experiments a German preparation ("Vogan") was used. The report of Chalier and Jeune shows that the French concentrate "Amunine" is capable of essentially the same effects and Vedder and Rosenberg verified Collazo's observations by using jewfish oil, a rich natural source of vitamin A.

These observations are important in showing that the toxic agent which most investigators have felt to be something other than vitamin A, is a rather common property of fish oil

concentrates. The opinion that vitamin A itself is responsible is based on two observations; the toxicity parallels the vitamin A potency and the destruction of vitamin A by irradiation also destroys the toxicity. Vedder and Rosenberg refute the first claim by showing that different jewfish oil preparations vary widely in toxic effect and that this is not related to the vitamin A potency. They believed to have demonstrated that molecular distillation eliminated the toxic substance or substances. Vitamin D and more strikingly 5 mgs. ascorbic acid effectively counteracted the harmful effects of jewfish oil.

The present status of the subject is that no final evidence has been produced to ascribe a toxic effect to vitamin A although certain concentrates, in large amounts, are distinctly harmful. Vedder and Rosenberg define this toxic range as being above the amount of concentrate containing 100,000 Int. Units of vitamin A, administered daily to rats weighing 50 grams or less.

#### MORBID EFFECTS IN MAN

The histological changes which follow deprivation of vitamin A in humans are indistinguishable from those experimentally produced in other mammals. However, species differences modify the location of the lesions, and the precise sequence in which they appear in man has not been established because the descriptions have so far been altogether too sketchy and few in number. Possibly differences exist in this respect. The disease in man differs from the experimental forms in that skin lesions seem limited to human cases. Even here the evidence is not definite enough to make a positive statement, and it is possible that careful search may show similar, or related, changes in the skin of experimental cases of the disease in question.

Including the case reported by Leber in 1883, thirty post mortem examinations have been recorded as well as various isolated lesions. In all but four of the autopsied cases, the subjects were infants. Following Leber's report came that of Wilson and Dubois in which a well advanced stage of deficiency must have existed since widespread lesions were found. In addition to the keratomalacia, which had been repeatedly described, typical epithelial lesions were found in the bronchi, pancreatic ducts, uterus, trachea, and submaxillary glands. The bronchial lesions had resulted in numerous bronchiectatic cavities and the pancreatic lesions in retention cysts.

The third recorded case was similar, and was reported by Thatcher and Sure. The patient was a breast fed infant (also

true of one case studied by Sweet and K'ang).

Subsequently, two series of cases have been published. Blackfan and Wolbach described the results of eleven post mortem examinations, including seven cases in which the diagnosis had not been made before death, but was established only when epithelial metaplasia was demonstrated histologically. Particular significance should be attached to this feature of their experience since it seems to promise that the diagnosis has frequently been overlooked, and that morbid effects of vitamin A poor diet are not uncommon in this country. The second series of cases has been described by Sweet and K'ang from Chinese subjects. In these the diagnosis had regularly been made before death from the presence of ocular lesions. One case had recovered from the effects of the deficiency before death from other cause, and another case was questionably one of deficiency since diphtheritic conjunctivitis was also present and masked the eye changes and no epithelial lesions were found at the autopsy. In six of their cases-including the treated one-no metaplastic changes were found though the search seems to have been thorough.

The bodies of victims of vitamin A deficiency are usually wasted. Abundant fat stores are rarely associated with the disorder (Blackfan and Wolbach). Retarded growth is very

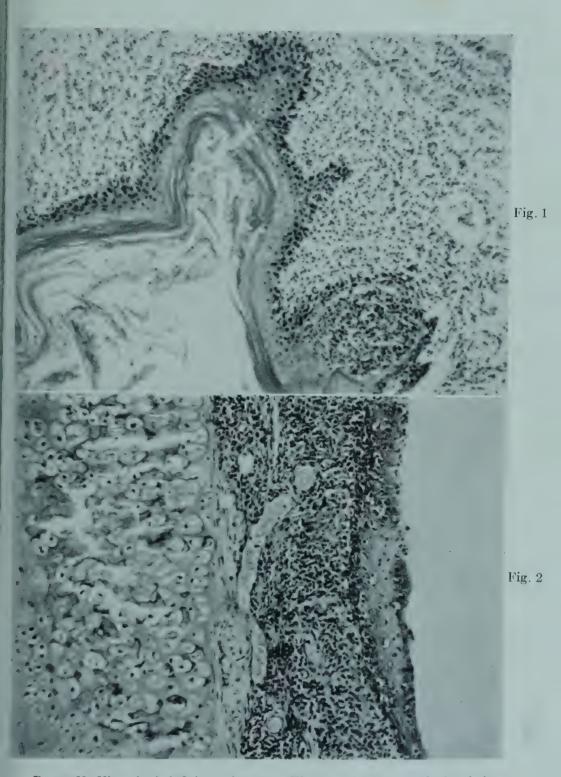


PLATE V. Vitamin A deficiency in man. Figure 1 shows metaplasia of the epithelium of the renal pelvis of a man who had been on a greatly restricted diet. The ureter was obstructed by epithelial debris. Figure 2 shows similar changes in the bronchial epithelium of an infant. Beneath the affected epithelium is a zone of inflammatory reaction, a common accompaniment of metaplasia in sites exposed to infection.



common, and atrophy of the lymphatic tissue and degenerative lesions of the skeletal muscles are found. The latter consist of swelling, loss of striation, and complete degeneration, often with calcification.

While the effect of lack of this particular food factor probably expresses itself in widespread changes in the body, the specific lesions are found in the eyes, conjunctival membranes, the epithelium lining various organs and ducts and the skin and its appendages.

## Ocular Lesions in Man

The ocular lesions commence as small, dry, round or triangular patches in the canthi—Bitôt's spots. They are formed of cornified epithelium and collect bacteria, very commonly bacillus xerosis. Cornification of the cornea is promptly followed by changes in the middle layers in which the cells become swollen and poorly stained and later vascularized. Infection, liquefaction and corneal destruction may then occur, sometimes extremely rapidly.

Similar changes occur in the conjunctival epithelium. The appearance of cornified cells is probably preceded by the involution of the mucus secreting elements. The conjunctival lesions are usually associated with congestion and hyperemia.

An early and prominent expression of the deficiency in the eyes, at least in adults, is a peculiar pigmentation which Pillat has studied in great detail, using various modern techniques for the demonstration of precursors of melanin. The pigment is preponderantly of epithelial origin and probably derived from the protoplasm of the corneal epithelium. It is a defensive reaction to the damage done by light to cells damaged by vitamin A deficiency. Pillat has repeatedly emphasized the general pigmentation in his cases, but this does not seem to have occurred except in China. He considers the skin pigmentation analagous to the corneal pigmentation though the adrenal glands may contribute. In a number of his cases, the

patients resembled cases of Addison's disease. The pigment is melanotic.

## Skin Lesions in Man

The skin lesions of animals have already been described. In man, dryness, scaliness, furunculosis, scalp abscesses and bleaching or loss of hair, may be present. The hair is also very dry. The follicular lesions, which are most helpful in establishing a diagnosis, vary in size to a maximum diameter of 5 mm. They are hard, deeply pigmented and surrounded by a zone of pigmentation.

The center of the lesion, the papule, is a scaly, pointed plug of keratinized epithelium which can be expressed leaving a huge crater. While comedones are common on the face and are distributed like those in acne, the keratinized lesions do not occur on the face, and the two are never associated, though both respond promptly to treatment with vitamin A. This suggests a lesser functional disturbance of the sebaceous glands of the face, and a more advanced organic lesion in the glands elsewhere.

Histological examination shows hyperplasia and hyperkeratinization of the related epithelium of the hair follicles with metaplastic changes of the sweat ducts and degeneration of the glands—accounting for the dryness of the skin. Occasionally the base of the hair follicle becomes cystic from retained epithelium and separates from the remainder of the shaft.

## Internal Organs

The most frequently affected internal organs are the trachea and bronchi and the pelvis of the kidney. In Sweet and K'ang's records, the trachea was found to have areas of metaplasia in six instances, the bronchi twice, and the pelvis four times. Other organs occasionally affected were: esophagus, three times; larynx, twice, and the uterus, tongue, pancreas

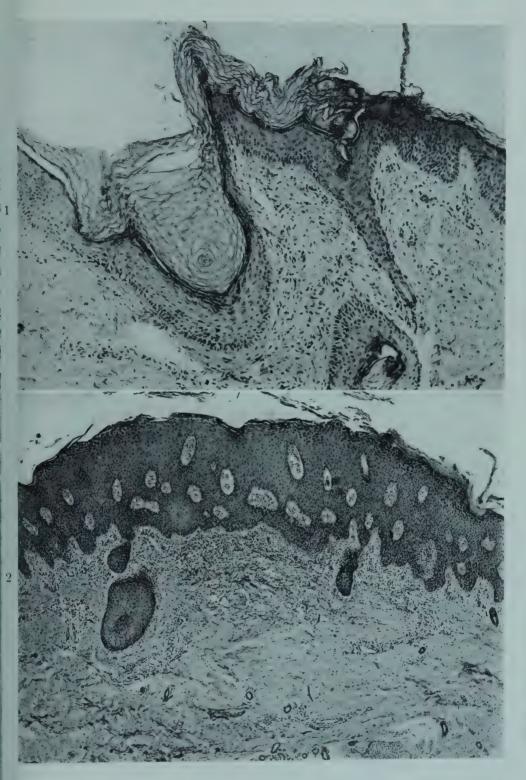


PLATE VI. Fig. 1. Extreme keratosis of the mouth of a sweat duct (right) and hair follicle (left) from a case of vitamin A deficiency. Fig. 2. Hyperplasia of the epidermis in a similar case. (Both photographs furnished us by Prof. C. N. Frazier.)

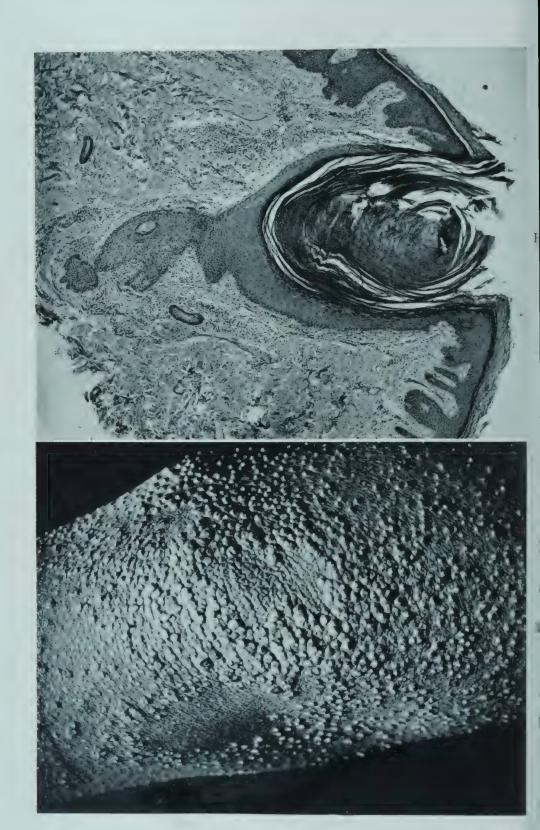


PLATE VII. Vitamin A deficiency in man. A keratotic plug in a hair follicle. The tip of the follicle is hyperplastic. Frequently the tip of such follicles becomes cystic. Fig. 2. A well developed keratosis of the skin in a Chinese subject probably due to deficiency of vitamin A. The photograph is of the elbow. (Both photographs from the collection of Prof. C. N. Frazier, Peiping Union Medical College.)

and prostate, once each. Blackfan and Wolbach found metaplastic changes in all their cases.

In one child the nares and accessory sinuses were affected. This was the only case in which these tissues were examined.

The histological structure of the lesions described by all observers of the disease in man are identical with those in experimental animals. There is no need, therefore, to recapitulate the description.

Hemosiderosis of the liver and spleen are commonly associated with vitamin A deficiency. Sweet and K'ang found these changes in 50 per cent of their cases. Blackfan and Wolbach also mention atrophy of the bone marrow.

No satisfactory explanation of the diarrhoea, which commonly occurs with xerophthalmia, has been given. Cramer describes profound atrophy of intestinal villi in experimental animals, but Sweet and K'ang found no analogous lesions in the human gastro-intestinal tract.

Pneumonia has always been a common associate of vitamin A deficiency. Of the seven cases in which Blackfan and Wolbach were able to recognize vitamin A deficiency only after autopsy, five had died of bronchopneumonia. Lobular pneumonia was present in eight of Sweet and K'ang's cases, and three more had pulmonary tuberculosis.

## Etiology of Epithelial Metaplasia

Epithelial metaplasia is not an uncommon lesion. It is frequently seen in cervical biopsies, for example, and in the respiratory passages adjoining tuberculous lesions. During recent years we have collected a considerable number of cases of metaplasia from surgical material alone. Such lesions are relatively common in the nasal passages, for example, where they constitute a characteristic feature of chronic atrophic rhinitis. Balo and Ballon report foci of metaplasia in the pancreatic ducts in material collected in Hungary. Warren found 9 such cases among 484 pancreases. In some the lesions

appear to have consisted only of heaped up masses of epithelial cells without keratinization. A case was reported by Haythorn in 1912. In the lungs were at least 12 patches of metaplasia. Haythorn states that he discussed the case with Klotz who showed him a pancreas in which the duct had undergone so advanced a metaplastic transformation that complete occlusion and cirrhosis occurred. Moore and Mark report 5 cases in human prostates. These were from patients with esophageal stenosis and probably represent genuine cases of vitamin A deficiency. In the casual samples which turn up in most pathological laboratories definite evidence of clinical deficiency is not present. At any rate that has been our experience.

Observation has taught that metaplasia often occurs at or near foci of chronic irritation or inflammation. In earlier days when intubation was commonly practiced for diphtheria the trachea sometimes became metaplastic about the cannula. The bifurcation of the trachea is a not uncommon site of metaplastic changes, associated, as a rule, with tuberculous lymph glands. The explanation for these lesions has not yet been found. Metaplasia has been occasionally produced by indwelling cannulae in the dog. It seems worth while to speculate on the possibility that these lesions all represent a disturbance of vitamin A nutrition. This theory has been tested in our laboratories in a number of experiments during recent years (McCullough and Dalldorf).

The information then available was that inflammation and irritation sometimes resulted in metaplasia; that stimulation with estrogen and vitamin A deficiency regularly produced metaplasia, the former only in the uterus. The association of metaplasia with inflammation in nutritional experiments had indeed been noted. In 1927 Goldblatt and Benischek stated that in rat experiments epithelial metaplasia seemed more severe if inflammation was present. We have found this is true, that if a silk thread is passed through the trachea of a

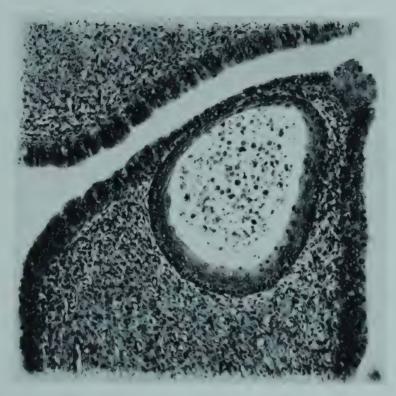
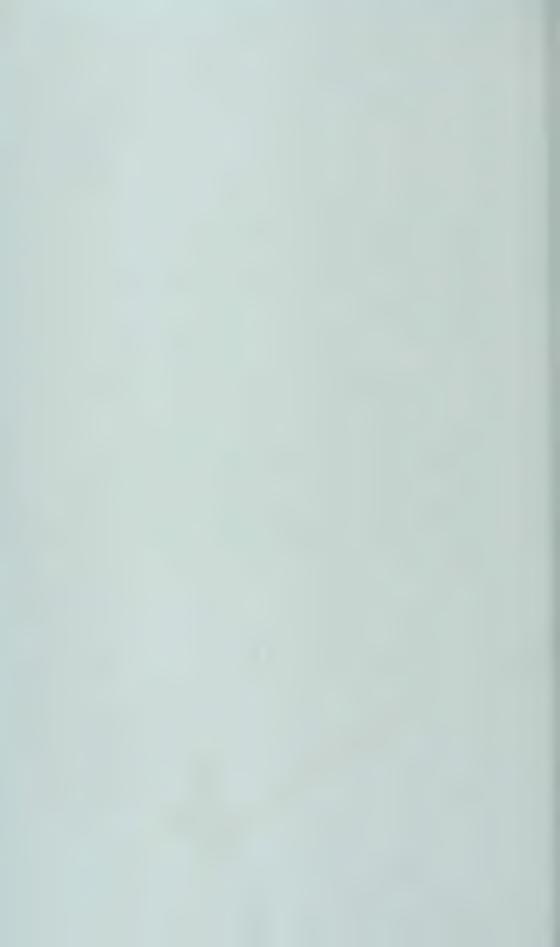


PLATE VIII. Effect of vitamin A on metaplasia induced by theelin. Photograph of the endometrium of a rat treated with theelin and maintained on a vitamin A deficient diet. Fourteen days before the animal was sacrificed it was given large doses of cod liver oil. The dilated gland has exfoliated keratinized epithelial cells. The basal layer of epithelium is extremely hyperplastic. This is the typical appearance of repair in vitamin A deficiency metaplasia and occurred despite the continued administration of theelin. (From McCullough and Dalldorf, Arch. Path., 24: 486, 1937.)



rat and the animal is then fed a vitamin A deficient diet metaplasia occurs by preference and more severely in association with the thread, or the lesion it induces.

The relationship between estrogen and vitamin A seems more complex. However we have found that if sufficient vitamin A be fed in conjunction with large doses of estrogen metaplasia does not occur, that the vitamin appears capable of preventing what is otherwise a very uniform, prompt, and extreme lesion. Sherwood, Depp, Birge and Dotson report that large amounts of carotene and vitamin A prevent the "normal" cornification of the uterus during estrus. They ascribed this to interference with the normal estrual cycle, to inhibition of the ovarian changes of estrus. The evidence for this interpretation is very inconclusive. It therefore seems that in the case of uterine metaplasia, induced by estrogenic hormone, as in trachea lesions incited by irritation, vitamin A plays the determining rôle.

Is the stage of cornification in the normal uterus and the metaplasia of estrogen overdosage to be considered a vitamin A deficiency lesion? If so it is purely a local one for the other epithelial surfaces are not affected. This conception, of a local deficiency, has been independently suggested by Choun.

Choun reported 5 cases of giant-cell pneumonia in infants. This is a rare disease which occurs in children between the ages of 6 months and 2 years. Clinically it resembles other forms of pneumonia but histologically the lesions are quite peculiar, consisting of giant cells in the alveoli and thickened, hyalinized alveolar walls to which the giant cells are connected. These Choun interpreted as metaplastic alveolar epithelium. Seifried's experience, to which reference has been made affords substantial evidence that giant cell formation may occur in the respiratory tract in association with vitamin A deficiency. In every case Choun was able to demonstrate metaplastic and hyperplastic changes in the bronchiolar epithelium as

well. Choun suggested that the inflammation might serve to produce "a local deficit in the vitamin."

It is true that in Blackfan and Wolbach's 13 cases of A deficiency in infants 7 were believed to have had an adequate diet and in 5 of these the suggestion was made that they might be due to a disturbed metabolism from chronic generalized infection. Such a mechanism could operate, through hepatic damage or other effects, to induce a systemic deficiency. But giant cell formation did not occur in any of these 13 cases. Choun's suggestion is an interesting one but the facts are still much too incomplete to justify any conclusions.

As far as estrogenic hormone is concerned there is other evidence that it is closely related. Zuckerman and Parkes produced metaplastic changes in the prostatic utricle of monkeys by estrogenic substance and recently Silberberg and Silberberg report that guinea pigs given large daily doses of estrogens develop skeletal lesions. The cartilage changes are described as "premature aging," the growing end of the bone is replaced by a bony plate. These changes, as far as can be judged from the illustrations and description, are very much like those of vitamin A deficiency. No mention was made of the condition of the epithelial tissues or the diet used.

#### THE DIAGNOSIS AND TREATMENT OF AVITAMINOSIS A

The large outbreaks of avitaminosis A have been associated with extreme privation, as among the poor of China, with wars and sometimes with special circumstances such as existed in Denmark during the First World War when the price of butter and milk rose to such outlandish levels due to the insistent demand by Germany for butter fats that the Danes stopped using milk products.

# Intake Not the Only Important Factor in Vitamin A Adequacy

More recently it has become obvious that dietary intake alone is not sufficient. Increasing interest attaches, therefore, to the studies of deficient absorption of vitamin A due to gastrointestinal and hepatic disease, and to increased requirements during periods of rapid growth and of severe infections.

Such secondary cases of vitamin A deficiency are being reported by various writers. Bloch saw xerophthalmia follow liver and pancreatic lesions. Blegvad saw a case secondary to carcinoma of the liver with occlusion of the bile duct. Pillat saw cases follow such severe intestinal disturbances as typhoid fever and dysentery. Wilbur and Eusterman saw a case of night blindness in a man who had developed a gastrocolic fistula after a gastric operation. Once the fistula was repaired the night blindness disappeared. Owen ascribed a case of xerophthalmia which appeared in a well fed patient to hepatic cirrhosis. One of Blackfan and Wolbach's cases developed deficiency because of congenital lesions of the biliary ducts.

The association of biliary imperforation—atresia—and vitamin A deficiency led Altschule to examine eleven cases of congenital atresia of the biliary passage for histologic evidence of vitamin A deficiency. All had received a liberal supply of vitamin A during life. Lesions were found in six cases. All but one of these were more than six months old, while all the cases without metaplasia were less than six months old. It may be assumed from this distribution that the deficiency was post natal, and it is extremely interesting that the exception was the infant of a woman who manifested symptoms of toxemia before delivery.

Altschule refers to the association of chronic jaundice and night blindness, xerosis and keratomalacia which was frequently commented on in the medical writings of the last century. These cases were undoubtedly similar to his own although they may have been exaggerated by dietary deficiency. At the time the observations were made the dietary nature of xerophthalmia was unrecognized and no dietary histories were given. The cases indicate, as do also more recent ones, that the association of jaundice and vitamin A deficiency is not a characteristic of infancy alone.

That faulty absorption is responsible for these secondary forms of vitamin A deficiency is shown by the studies of Blegvad in which vitamin A administered intramuscularly, but not orally, cured the symptoms of the deficiency. It has also been demonstrated both clinically and experimentally that biliary fistulae induce vitamin A deficiency irrespective of the diet. In the experimental studies (Greaves and Schmidt) the necessary amounts of vitamin could be supplied orally if given with bile salts. Therefore, two methods of administering vitamin A are available for patients suffering from deficiency due to lack of bile in the small bowel, either parenteral or oral in combination with bile salts.

An interesting discovery was reported by Breese and Mc-Coord who studied the vitamin A absorption in cases of celiac disease. A large test dose of vitamin A was given by mouth and the blood vitamin A determined at 2, 4, 6, 9, 12 and 24 hours. Normal absorption is sufficiently rapid to produce a peak concentration within 4 hours. In 10 tested cases of celiac disease absorption was much reduced and delayed.

Diabetes mellitus is often associated with hypercarotenemia which is thought to be due to an inability of the liver to convert carotene to vitamin A. Thus Ralli and her associates have demonstrated that diabetics not only have higher blood concentrations of this pigment but that they cannot handle excessive amounts as effectively as non-diabetics do. Brazer and Curtis examined juvenile diabetics by means of the Biophotometer, testing the influence of both carotene and vitamin A on their adaptation. They found evidence of poor adaptation to be very common and several of their patients gave histories of night blindness as well. Carotene did not correct this defect but vitamin A did indicating that conversion was abnormal. Thus the diabetic may become deficient in vitamin A because he is unable to convert provitamin A into the active form.

#### AGE GROUP REACTIONS

The disease occurs in all age groups and has been seen in the new born as well as in persons of advanced age. The majority of cases occur in infants, however. The precise period of infancy varies with the dietary habits of the people. In the Danish epidemic the infants were bottle fed on skim milk and the average age was less than in the Japanese cases in which the deficient diet was given after weaning. Among the 203 cases reported by Sweet and K'ang a second peak of incidence occurs in early adult life. This is ascribed to the impoverished diet prevalent among soldiers and apprentices in China. Cases secondary to gastro-intestinal or biliary lesions may, of course, occur at any age although the requirements of infancy are apt to be more imperative and relatively greater.

The epidemics of vitamin A deficiency in Europe during the World War occurred mainly in the winter months, Birch-Hirschfeld's cases appearing between November and February. The peak incidence in China occurs during the same season although a smaller rise was found in summer which was attributed to dysentery and consequent depletion.

Judged by the information we now have the epidemics of vitamin A deficiency have been predominantly marked by eye or skin lesions. In some instances the separation has been so sharp that one might doubt whether the two are related. Yet in many instances this has been only a matter of emphasis and in certain cases both manifestations have been present. Most of Frazier and Hu's patients who had keratomalacia also had skin lesions although the reverse was not true. Of Loewenthal's 81 cases of deficiency 74 had dermatosis, 45 had xeropthalmia and 71 hemeralopia.

The medical history usually reveals diets which have been extremely poor, or completely lacking, in animal fats and green vegetables, either of the patient or, if a breast fed infant, of the mother.

The incubation period is variable, depending on the degree

of deficiency and the previous intake of the vitamin. It also varies with the age of the patient. Forest and Wolff estimated the depletion period in very young infants as three to four months, while twice as long a period has occurred in children more than twelve months old.

Infant cases are usually stunted, with a dry scurfy skin less sensitive than normal, sometimes with loss of hair and frequently with skin infections. Diarrhoea is common and many cases have come under observation because of complications, usually bronchopneumonia. In Ceylon, the affliction is called "mandama" and the four characteristics are said to be xerophthalmia, stunted growth, diarrhoea, and a toad-like skin which Nicholls calls phrynoderma.

Bloch said it was rare for a child to appear well and develop xerophthalmia though occasionally an infectious disease seemed to precipitate it. The children were first indolent and then irritable. Anemia and latent or manifest edema are common.

## Night-blindness and Xerophthalmia

The first definite symptom is night blindness—hemeralopia. This is seldom recognized in very young children and it sometimes develops so insidiously that it is recognized late in adults. Patients complain that they are unable to get about in darkened, but familiar surroundings. In a recently recorded case, the patient had complained to the civil authorities that the street lights were defective. Exposure to strong light aggravates the defectiveness of vision in poor light, and vision is better in the morning than in the evening because of exposure to light during the day.

An extensive study of mild cases of hemeralopia has recently been made by Fransden and is of interest because of his analysis of the symptoms present in such cases. Common complaints were difficulty in sewing and reading at night unless the light was brilliant, dancing of letters on the page, poor vision exaggerated by weak light, photophobia, glittering images, and dancing specks—muscae volantes. Less frequent symptoms were altered sensitivity—paraesthesias—, muscular twitchings of the eyelids and extremities, nervousness, dryness of skin and mouth, pelvic pains, and decreased sweating. We may assume from this, that some of his patients had other symptoms of A deficiency than the hemeralopia alone.

The signs of the disease in the eyes are a patchy dryness and Bitôt's spots. These latter resemble dried foam, are gray or light yellow in color, impervious to tears, and look as though they might easily be brushed off. Loss of luster and wrinkling of the conjunctiva follow. Conjunctivitis and photophobia are often associated with the early lesions, and in some cases mask the more important changes. A light brown pigmentation throughout the conjunctiva is also characteristic as is reduced sensitivity of the conjunctiva and cornea, which may persist for months after all other signs have disappeared. both eyes are involved and at about the same time. In older children a viscid, stringy conjunctival secretion is present and the lids are stuck together. When this is cleaned away the cornea may still be clear but photophobia and blepharospasm are complained of. As the disease advances the Bitot's plaques appear and may cause adhesions between lid and eyeball (Yudkin). One or more small ulcers may occur on the cornea and these progress to perforation. However, older children usually have only a xerosis conjunctivae. Hypopyon is characteristic of the disease in infants at which time of life the lesions develop rapidly.

These manifestations are all dependent on the fundamental alteration in the surface cells which precedes the macroscopic changes. The Bitôt's spots are areas of thickened epithelium; the conjunctivitis is dependent upon degeneration of the mucus secreting cells, the roughening of its surface which harbors bacteria, and probably impaired tear glands and ducts which

reduce the cleansing action of tears.

If the condition is allowed to progress the cornea becomes

gray and opaque and softens. These lesions may be solitary, but in other cases many small foci liquefy simultaneously. An effusion of pus—hypopyon, prolapse of the iris, and even general inflammation of the eyeball—panophthalmitis—are late effects.

The progress of the eye lesions is less rapid in adults in conformity with the essential differences in other aspects of the disease in the young and in the full grown. Blindness is a common result of vitamin A deficiency and, particularly in the young, treatment must be begun early in the disease before corneal opacity occurs.

## Skin Lesions

Current interest in the skin manifestations of vitamin A deficiency may be credited to the investigations of Nicholls in India, Loewenthal in Africa and Frazier in China. Nicholls and Frazier both deduced that the dermatosis was a very early sign of deficiency, an observation in good accord with later evidence. Nicholls found advanced cases without evidence of ocular disease and Frazier and Hu reported cases in which the skin lesion was seen to precede eye signs by several weeks. The most complete description has been supplied by Frazier and Hu.

The earliest change is an abnormal dryness of the skin due to suppression of the sweat glands. This is followed by keratosis which is most extensive in the hair follicles which become filled with dry, hard, pointed papular masses of keratinized epithelium and may be very irritable. These lesions are commonest on the antero-lateral aspects of the thighs, the postero-lateral aspects of the forearm and gradually extend to the extensor surfaces of the shoulders, the lower abdomen and sometimes to the chest, back and buttocks. The papular masses are of a dark, dirty color and are frequently surrounded by a zone of greyish pigmentation. The skin between is dry and roughened. Various observers have remarked that the

condition can be more easily recognized by palpation than by inspection. The scaliness gives a whitish coloration to the skin of negroes who have said they "ash" (Lehman and Rapaport).

Frazier and Hu found age to be a determining factor in the appearance of these skin lesions. Of the cases of vitamin A deficiency observed in the Peiping Union Medical College only 2 per cent of the cases of keratomalacia under 15 years of age had dermatosis while 30 per cent of the adult cases had characteristic lesions. They felt that before puberty vitamin A deficiency results in a condition of simple xerosis, after puberty in xerosis and spinous follicular lesions. This would explain the absence of similar lesions in the Danish epidemic which was limited to infants. However Aykroyd and Rajagopal found 129 typical cases among Indian children of school age and one fourth of the 4,380 children examined by Nicholls had similar lesions. Xerosis is more common but follicular lesions also occur in children. It seems reasonable, although by no means certain that those instances of keratomalacia without follicular lesions represent a more severe and acute deficiency, exaggerated by the more insistent demands of rapidly growing infants and that the follicular lesions require a considerable time to evolve just as they are very slow to heal.

In certain cases lesions have also been seen on the face. These superficially resemble acne. Youmans and Corlette, in describing 6 patients they observed in Nashville, remark that in addition they found other patients with lesions having the same distribution and essentially the same microscopic structure but associated with an inflammatory reaction. Many of the individual lesions looked pustular or acneform although no frank exudate could be expressed from them. The lesions were not as elevated as the spinous ones described by Frazier and Hu and the skin was not dry. Nevertheless these atypical lesions responded to treatment with vitamin

The evidence of night blindness and of xerosis conjunctivae was indefinite in Youman and Corlette's cases. This was interpreted to mean that the skin lesions preceded the eye signs. The significance of the acneform lesions is still uncertain. They were not uncommon in Loewenthal's cases for he wrote that the skin "presented the clinical picture of acne vulgaris combined with a dermatosis which none of the medical officers present could define." Loewenthal's solution of this problem is all the more creditable because his cases were complicated by other deficiencies. Neuritis, itchy scrotum, mouth lesions, diarrhea, general infections, cutaneous sepsis and changes in the hair. These he distinguished from the cardinal signs of vitamin A deficiency, xerophthalmia, keratomalacia and night blindness. "At subsequent monthly inspections of prisons, all new cases of this dermatosis were recorded, and it was found that the majority of these men suffered from night blindness and xerophthalmia, while almost every sufferer from xerophthalmia and night blindness showed these cutaneous changes."

Thus evidence concerning the striking character of the skin changes associated or not associated with xerophthalmia was accumulating and older reports took on fresh meaning. Nicolau's report of keratotic lesions with associated acneform ones having the distribution and minute structure of Frazier and Hu's, Loewenthal's and others cases may now be interpreted as due to vitamin A deficiency. Nicolau ascribed these cases to scurvy, they occurred in Roumania during a scurvy epidemic (1918) and were sometimes associated with petechiae. Aschoff and Koch, in their classic study of the same epidemic, dismissed the idea that they were scorbutic lesions. "These elevations have nothing to do with scurvy." The lesions were well known, according to Aschoff and Koch, to the Turks who called them "keratosis pilaris" and who reported that they were very common in Turkey. Aschoff and Koch designated these lesions as keratosis suprafollicularis and described

them in sufficient detail to establish their identity with those of Frazier and Hu. They were frequently seen in patients without a single sign of scurvy. In this regard it is interesting to note, nevertheless, that according to earlier writers quoted by Aschoff and Koch, the lesions had long been considered an early manifestation of scurvy.

Keil has recently reported 2 cases of dermatosis associated with scurvy. One patient had ulcerative colitis, positive capillary fragility test and responded clinically to vitamin C. Her skin was generally very dry and hyperkeratotic areas occurred on the arms, thighs and abdomen. The hairs in the diseased follicles were fine, deformed and curly. Each lesion was surrounded by an area of hyperemia or of hemorrhage. The second patient had been on a grossly deficient diet. The lesions were identical. Keil refers to the older references to "lichen scorbuticus" or "scorbutic goose-flesh" and suggests it is a genuine manifestation of scruvy. It is not as marked as the keratotic dermatosis of vitamin A deficiency. Neither patient gave symptoms of night blindness. These cases seem to us to represent deficiency of both vitamin A and C. There is no justification in the known facts of the pathogenesis of scurvy to ascribe keratotic and metaplastic lesions to it while these changes are typical of vitamin A deficiency wherever it occurs.

More pertinent are the instances of typical dermatosis reported from our own country. Jeghers described lesions of the same type although of less severity among medical students and Steffens, Bair and Sheard produced the lesion in a healthy individual by vitamin A depletion. More recently Lehman and Rapaport have identified 9 cases among children in New York City. Their report is of particular interest in two respects. In the first place they followed all of their cases with photometric tests for visual adaptation using the Biophotometer. In each patient subnormal readings were secured although ocular lesions were not recognized. In 4 of

their patients these observations were fortified by determinations of the immediate response to large doses of vitamin A (200,000 Int. Units given orally). In 2 a marked improvement was detected within 2 hours. The second noteworthy feature of the cases of Lehman and Rapaport is that the distribution of the cases within the families suggested a hereditary influence. In one instance 2 sisters both had dermatosis while the parents and 3 other siblings were apparently normal although the mother had suggestive evidence of night blindness. In a second family the mother and 2 daughters had had the skin lesion for many years while the father had a normal skin.

## Other Symptoms

Hoarseness and a dry cough often occur and are probably due to reduction in the bronchial mucus secretion. Diarrhea is also common. Paraesthesia and motor disturbances have been reported but are presumably due to other causes. Of Pillat's cases 40 per cent had gastric hypoacidity and Pillat judged this was related to both the severity and the progress of the deficiency.

The common complications are pneumonia, pyelitis and cystitis. Herbert found desquamated epithelial cells, and often pus cells as well, in the urine of all of his cases.

## Prognosis

Prognosis naturally varies with the severity and duration of the deficiency. In infants the mortality rate has always been high during epidemics of xerophthalmia. During the Danish epidemic it was 21 per cent. The incidence of blindness is, of course, much higher and stigmata of the disease, which affects life expectancy, remain after recovery. In Bloch's patients, many of whom were kept in homes and institutions for blind children where their progress could be observed, it was found that one-third died before their 8th year. The milder cases without eye lesions recover promptly under proper treatment.

#### TREATMENT

The treatment consists in giving vitamin A in adequate amounts in the diet and by use of concentrated preparations. Cod and halibut liver oils, A concentrates and carotene, are all suitable. In cases where an obstructive jaundice co-exists, bile salts must be given with the vitamin for its absorption depends upon their presence.

Since human cases have almost invariably developed after diets deficient in several factors, a well planned, complete dietary routine is desirable. Vitamin A deficiency rarely occurs singly. Of the recorded cases in infants, the most common associated deficiency disease has been rickets, but perhaps that is only because symptoms of other vitamin deficiency were not recognized.

It seems likely that cases may be found in which the conversion of carotene into vitamin A is interfered with by liver disease, but use of carotene has been successful in most cases. Such limitations will be few. Faulty absorption of the vitamin and of its precursor, the factors making for further inhibition or lack of utilization, need much further study.

Wald, Jeghers and Arminio, measuring the threshold of the completely dark adapted eye, were able to correlate almost immediate response (7 minutes) to vitamin A given parenterally. Fisher reports a severe case of night-blindness in a boy. A single dose of 36,000 Int. Units of vitamin was given and the night-blindness responded within one hour. The conjunctival sacs became moist and distinctly improved within a week. Treatment was supplemented with cod liver oil instilled into the conjunctivae. Similarly Aykroyd and Wright reported children with keratomalacia showed arrest of the lesion in 1 week and definite improvement within 2 weeks. Vaillant and Gillis reported that a young girl almost completely blind at night given 10,000 Int. Units daily improved in several weeks.

The skin lesions respond more slowly. Youmans and Corlette seldom saw response within 1 month. Often 3 months

was required. However Frazier and Li, who treated a young male adult with 1 to 2 mgm. of carotene daily (given intramuscularly) observed shrinkage in the papules within 1 week. By the 9th day many plugs were falling out of the gaping follicle mouths and a corneal ulcer began to heal. Three days later it was completely healed. The man's skin was much smoother within the month although a few lesions could still be found on discharge, 51 days after treatment was commenced. Nicolau's cases, treated by an adequate diet alone recovered within 3 months although pigmented areas remained. This has been noted by subsequent authors. Lehman and Rapaport's patients showed a very prompt response to treatment, as measured by the photometer test but required 2 months for resolution of the dermatosis.

# Special Aids in the Diagnosis of Vitamin A Deficiency

The special tests which have been suggested have been chemical measurements of the vitamin of the blood, methods of establishing the presence of metaplasia and means of identifying and measuring night blindness.

Night blindness can usually be determined from the patients history if well developed. Simple procedures such as the use of a watch dial with luminous numerals will frequently confirm the diagnosis. In addition various special instruments have been designed and used for measuring the degree of dys-adaptation. Most of these are expensive and their use requires special training. All operate on the principle that if the eye be first exposed to a brilliant light and the retinal pigments bleached the rate of recovery may be determined by establishing the threshold at which targets of variable brilliance may be identified. The first instrument of this type, the Birch-Hirschfeld photometer, was relatively simple, a diaphragm being used to regulate the intensity of the illumination of the target. The bleaching light was not provided. The values were purely arbitrary.

A popular successor to this instrument was the Biophotometer which incorporated the bleaching light within it and also

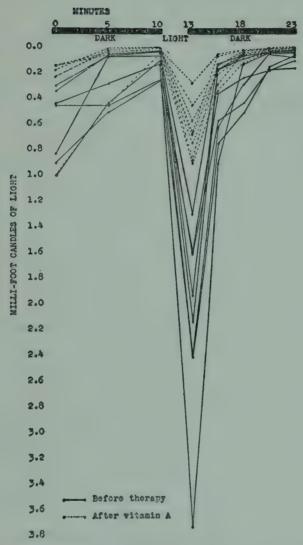


Fig. 19. The results of visual adaptation tests made with the "Biophotometer" on eight children before and after vitamin A therapy. Other signs of vitamin A deficiency were present in these cases. (Reproduced by permission of Dr. Edw. Lehman and the J. A. M. A. See Lehman, E., and Rapaport, H. G., J. A. M. A., 114: 386, 1940.)

attempted to express the intensity of the illumination in appropriate values. To much of the work with both these

instruments the objections were raised that many patients learned to read the targets in dimmer light quite independent of their actual adaptation and also that the changes reported after treatment could equally well be explained by chance.

The phenomenon of dark adaptation was then thoroughly studied by Hecht and an improved instrument devised which tests a fixed retinal field (necessary because the proportions of cones and rods in different regions of the retina vary and therefore the response to dark adaptation tests) and incorporates superior control of the light values used. This machine has not been widely used but appears to afford the most precise means of determining and measuring dark adaptation.

However subsequent work has shown that the simpler instruments will also yield significant observations if their limitations are thoroughly controlled. Jeghers' results seem to be reliable and extensive work by Booher and Williams is encouraging. These studies were made with the Biophotometer. Even simpler instruments are satisfactory. Pett has described a simple device which he found suitable. We have used a small instrument (described in the Appendix) with good results. The reader should consult the reports of Hecht and Mandelbaum, Jeghers and Pett.

The diagnosis of manifest cases of vitamin A deficiency can be made more certain if certain special examinations are made. According to Sweet and K'ang, the best procedure is to retract the eyelids for three to five minutes, watching all the while for the appearance of dullness or haziness, and then to wipe a spatula lightly across the conjunctiva and stain the cells secured. Keratinized epithelium and bacilli xerosis will be found early in vitamin A deficiency. This method is particularly suitable for infants, since it requires no cooperation from the patient.

Blackfan and Wolbach recommend the following criteria:

<sup>1.</sup> An inquiry into the food habits of the patient, particularly the supply of vitamin A containing foods. The tabulation of vitamin A potency of

common foods in the Appendix will be found helpful in estimating such dietary adequacy.

- 2. Examination for morbid processes which might interfere with the use of fats, such as diseases of the biliary system and pancreas, vomiting or diarrhoea.
  - 3. Examination of the eyes for xerosis or night blindness.
- 4. Search for keratinized epithelial cells in scrapings from cornea, nose and mouth, the secretions of the vagina or in the urine. They recommend staining by Gram's method with acid alcohol decolorization. Sweet and K'ang felt the distinction between the buccal epithelium in health and deficiency was difficult to determine in scrapings.

Youmans and Corlette made and examined smears from the bulbar conjunctiva of normal and poorly fed individuals in an effort to establish criteria for the diagnosis of mild forms of deficiency. The cells were finally classified as nucleated or non-nucleated and the results showed no significant difference between the proportions of each type and the nutritional status of the patients. In none of the cases studied were definite signs of vitamin A deficiency present but several of the cases were believed to represent partial deficiencies. It seems apparent, therefore, that this criterion of deficiency is inadequate in partial states of depletion as we have been recently accustomed to think of them or will require an improved technique. That it is satisfactory in the diagnosis of cases with signs of the disease is well attested.

The determination of vitamin A concentration in the blood and urine has been used clinically. In these tests the usual procedure has been to use the antimony trichloride method described in the Appendix or a modification of it. The results naturally suffer from the well known weaknesses of the test which does not measure the vitamin A value at different levels of concentration with any considerable accuracy. However it is useful to the degree that cases of severe depletion, as reported by Haas and Meulemans, demonstrate a complete absence of blood vitamin A. Serum carotene, which is simply and accurately measured seems to bear no close relationship

to the vitamin A value and carotene was present in all of the samples tested by Haas and Meulemans. Quantitative deductions from serum vitamin A do not seem to be justified.

The presence of vitamin A in the urine has not been correlated with vitamin A nutrition. The vitamin is present only during diseases which affect the liver and might be considered more a test of liver function than of vitamin A nutrition. Vitamin A spill does not occur in the urine even after massive doses unless the liver be damaged (Schneider and Weigand). Wendt has improvised a tolerance test in which the intestinal elimination of vitamin A is measured and used as a yardstick to determine when saturation has occurred.

# Thyroid Gland and Vitamin A

An association between the thyroid gland and vitamin A was suggested more than 10 years ago. Rabinowitch reported that the action of iodine in hyperthyroidism could be increased by adding a small amount of vitamin A. Fraser and Cameron confirmed this the same year. Extensive studies were reported the following year by Abelin who supplied various confirmatory evidence such as the increased vitamin requirement in animals given thyroxin. Later Wendt declared these two agents are antagonistic and reported that hyperthyroidism could be successfully treated by cod liver oil alone. Cases of exophthalmic goitre were shown to have abnormally small amounts of vitamin in their blood plasma and these returned to normal after thyroidectomy. Wendt's handling of these cases included the use of 50,000 to 80,000 units of vitamin A t.i.d. and blood determinations to insure a high level (5 to 10 Lovibond blue units per 10 cc. blood). Schulze and Hundhausen added experimental studies which confirmed the general belief that thyroxin increased the vitamin A requirements.

There are some reasons to believe that the formation of vitamin A is dependent on thyroid function. Thus removal

of the thyroid gland of goats causes the milk to become pigmented (carotene).

# Use of Vitamin A in Wounds

Repeated clinical endorsement of vitamin A rich ointments in the treatment of skin wounds including burns have been made. The effectiveness of these preparations is ascribed to the vitamin A they contain since irradiation (and destruction of the vitamin) diminishes their power. Löhr's reports have been especially enthusiastic. In industrial wounds he claimed that cleansing followed by the application of a thick paste rich in vitamin A usually insured complete healing within 14 days. Skin transplantation was avoided, the epithelial regeneration strikingly active. A third degree burn 45 sq. cm. in size was completely epithelialized by this means. Puestow, Poncher and Hammatt verified this in experimental burns. The rate of healing was 25 per cent faster if cod liver oil ointments were used. Epstein also noted some increase in rate of healing but the results did not seem permanent. One of his patients developed dermatitis from the preparation used.

Vitamin A is capable of a direct effect on the growth of epithelium as tissue culture studies in our laboratories have shown. It is known, too, that local application is helpful in treating xerophthalmia. Loehlein used a salve containing vitamins A and D for various traumatic and inflammatory lesions of the cornea and conjunctiva. The results were encouraging.

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## CHAPTER VII

## THE NATURE AND FUNCTION OF VITAMIN B1

As noted in Chapter II, vitamin B<sub>1</sub> has now been shown to consist of a combination of a pyridine group with a thiazole group and this has been confirmed by synthesis. The availability of the synthetic product has made possible a study of the behavior of this vitamin without complication with other vitamin factors.

Fundamentally, it is now demonstrated that when vitamin B<sub>1</sub> is combined with phosphoric acid it functions in carbohydrate metabolism in the tissues as a co-carboxylase and this particular carboxylase system brings about the breakdown of pyruvic acid into CO<sub>2</sub> and water. The experiments of Peters at Oxford first demonstrated this effect by comparison of the behavior of brain tissue of vitamin-deficient and vitaminadequate pigeons. Peters showed that in the absence of adequate vitamin B<sub>1</sub> there was an accumulation of both lactic and pyruvic acid in the brain tissue and he also showed that minced brain tissue from the deficient birds could have its oxygen uptake restored by adding B<sub>1</sub> in in-vitro experiments.

Platt and Lu were the first to demonstrate that in classic beriberi cases there was an increase in pyruvic acid in the blood and such increase has now become a means of diagnosing vitamin B<sub>1</sub> deficiency since this pyruvic acid can be measured by combination with bisulfite. Löhman and Schuster provided the further confirmation of this general function of vitamin B<sub>1</sub> by isolating the cocarboxylase from yeast and showing that it was the phosphorylated thiamin molecule.

At one time it was thought that disturbances such as polyneuritis might result from the dumping into the system of

lactic and pyruvic acids in excess. Peters, however, inclines to the viewpoint that mere failure of normal carbohydrate metabolism in the tissues would in itself be sufficient to account for the dysfunction following vitamin deficiency.

The absence of an important factor in the development of energy from carbohydrates would be sufficient to stop the normal functioning of some group of nerve cells. Those which normally had most work to do might be expected to run out of their supply of catalyst B<sub>1</sub> sooner than others.... Several circumstances may lead to these developments in pigeons, most of which can be considered to be interference with some stage of sugar metabolism; we may mention asphyxia, insulin overdose, cyanide poisoning, and anaesthetics such as chloroform. This is particularly interesting in view of similar analysis carried out independently and at the same time by Quastel and colleagues upon the influence of narcotics. They found that narcotics influence the lactate oxidations more than the succinate.

As a result of interference with the metabolism of the cell, we get failure of function. It is not necessary to invoke the idea of a toxic agent unless this view was extended to include water in the wrong place in a cell!

## RELATION TO CALORIE AND NUTRIENT INTAKE

Funk (1914) by contrasting diets containing a high percentage of carbohydrate with others high in fat or protein showed that the onset of polyneuritic symptoms came much earlier on the high carbohydrate diets. This was the first suggestion that vitamin B<sub>1</sub> needs are related to fuel supply and to carbohydrate fuel in particular.

Cowgill confirmed the relation of B<sub>1</sub> requirement to calorie intake by a series of studies with different species of animals. He found that given the weight of the animal and the calorie intake one could express vitamin B<sub>1</sub> need by a formula which

may be written as follows:

# $\frac{\text{Vitamin B need in milligram equivalents}}{\text{Calorie Intake}} = \text{K} \times \text{Weight}$

In this formula K is a constant of definite value for each species of animal and Cowgill's milligram equivalents have been shown to be  $\frac{1}{20}$  of an International Unit of  $B_1$  or  $\frac{1}{20}$  of

0.003 mgm. of thiamin. For the human species this formula becomes:

Thiamin needs in Int. Units = 0.00142 × weight in kilograms × calorie intake

If we multiply this value by 0.003 we may express the requirement in actual weight of thiamin in milligrams.

Jolliffe (1938) worked out table 19 with the use of this formula.

TABLE 19 (After Jolliffe)

| BODY WEIGHT |      | B1/CALORIE<br>RATIO | INT. UNITS B1 REQUIRED WHEN DIET SUPPLIES FOLLOWIN CALORIES: |      |      |      |      |
|-------------|------|---------------------|--|------|------|------|------|
|             |      |                     | 1500   | 2000 | 2500 | 3000 | 3500 |
| kgm.        | lbs. |                     |  |      |      |      |      |
| 40          | 88   | 1.20                | 90   | 120  | 150  | 180  | 210  |
| 45          | 99   | 1.33                | 100  | 133  | 166  | 200  | 233  |
| 50          | 110  | 1.47                | 110  | 147  | 184  | 220  | 257  |
| 55          | 121  | 1.62                | 121  | 162  | 202  | 243  | 283  |
| 60          | 132  | 1.77                | 134  | 178  | 222  | 266  | 311  |
| 65          | 143  | 1.91                | 143  | 191  | 239  | 286  | 333  |
| 70          | 154  | 2.05                | 154  | 205  | 256  | 307  | 359  |
| 75          | 165  | 2.22                | 166  | 222  | 277  | 333  | 390  |
| 80          | 176  | 2.40                | 180  | 240  | 300  | 360  | 420  |
| 85          | 187  | 2.53                | 190  | 253  | 316  | 279  | 443  |
| 90          | 198  | 2.65                | 200  | 266  | 332  | 398  | 464  |
| 95          | 209  | 2.80                | 210  | 280  | 350  | 420  | 490  |
| 100         | 220  | 2.95                | 221  | 295  | 369  | 442  | 516  |
| 105         | 231  | 3.10                | 232  | 310  | 387  | 465  | 542  |
| 110         | 242  | 3.25                | 244  | 325  | 400  | 487  | 569  |

Of this formula and the values obtained with it Cowgill has the following to say:

It should be emphasized that estimates of the human requirement for vitamin B derived from my formula pertain to the minimum or beriberi preventing level; the optimal intake is undoubtedly much greater.

The limitations of this formula are discussed in greater detail in Chapter IV. It is generally conceded, however, that

if the criterion of need be amounts necessary to prevent visible symptoms of B<sub>1</sub> deficiency Cowgill's formula constitutes a quite reliable prediction estimate.

The formula may also be written in the following form:

Thiamin need in I.U./calories = 0.00142 × weight in kilograms

The thiamin/calorie ration may be taken as an index of vitamin B<sub>1</sub> adequacy. Williams and Spies consider that a B<sub>1</sub>/calorie ratio of 1.7–2.29 is a borderline ratio; below 1.7 indicates danger of beriberi and above 2.29 safety from beriberi. Cowgill has constructed a chart of thiamin/calorie ratios in regard to weight which also gives ratios above and below which there is safety or danger.

From his chart, it would seem that calorie intake regardless of source affects vitamin B<sub>1</sub> need. Jolliffe and co-workers established such a relationship rather strikingly in noting the effect of alcoholism on the development of polyneuritis. They reported on 42 alcohol addicts who gave reliable dietary histories. Twenty-six of these had polyneuritis. When the diets of 14 of these polyneuritics were analyzed they gave a B<sub>1</sub>/calorie ratio of 2.7 or more but when utilizable alcohol calories were added to the diet calories the ratio fell to 1.7 or below. They have found that a ratio of 1.7 or below in human dietaries is invariably associated with high incidence of beriberi.

Does it then, make any difference from what source the calories come? Funk's 1914 experiment indicated that increase in carbohydrate intake increased B<sub>1</sub> needs and a shift to fat was sparing of B<sub>1</sub>. Others reported similar observations in more recent times. Williams and Spies have reported considerable evidence to support the view that only non-fat calories should enter into the prediction B<sub>1</sub>/calorie ratio.

To check this point Jolliffe recalculated 100 of Cowgill's dietaries associated with and unassociated with beriberi and determined the following ratios: thiamin/calorie; thiamin/

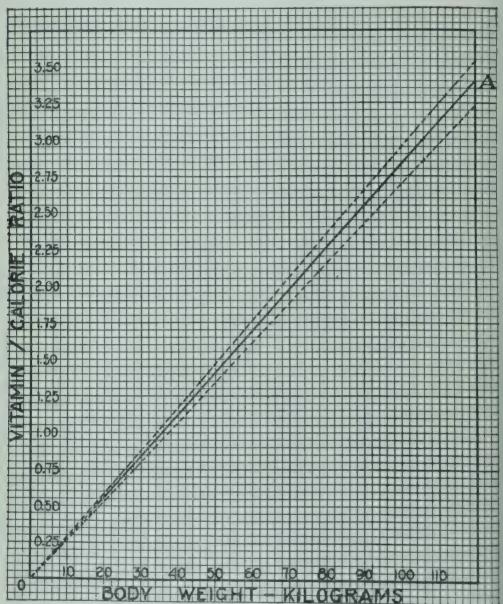


Fig. 20. Cowgill's prediction chart for estimating the vitamin B adequacy of any given diet. "The plot indicated by line OA represents the probable minimum vitamin B(B<sub>1</sub>) requirement referred to body weight. The area between the dotted lines represents a zone of uncertainty. If the Vitamin/Calorie value of the diet for a given body weight falls definitely above line OA, the ration is deemed adequate with respect to vitamin B; if the plot proves to be appreciably below the line, the vitamin requirement is not satisfied by this diet and beriberi should occur provided the period of subsistence on this ration is sufficiently extended; if the plot is close to line OA, or between the dotted lines, the diet may be considered as 'borderline' in character.' (Reprinted by permission of the author.)

carbohydrate calorie; thiamin/non-fat calorie; B<sub>1</sub>/calorie ratio of Cowgill. His results are shown in table 20.

Since in this series Cowgill's B<sub>1</sub>/calorie ratio gave lowest per cent of error and thiamin/carbohydrate calorie highest error; since also the thiamin/non-fat calorie ratio was also productive of higher error than the B<sub>1</sub>/calorie ratio, Jolliffe feels that it is not necessary to consider source of calories in the prediction estimates.

There is also some controversy over the significance of weight in the Cowgill formula. Williams and Spies question whether weight produces so great a difference in B<sub>1</sub> need as the formula would indicate and incline to the view that the

TABLE 20 (After Jolliffe)

| PREDICTION INDEX                        | NUMBER<br>OF<br>DIETARIES | IN ERROR | PER CENT<br>ERROR |
|---|---------------------------|----------|-------------------|
| Thiamin/calorie ratio                   | 85                        | 4        | 4.7               |
| Thiamin/carbohydrate calorie ratio      |                           | 3        | 4.8               |
| Thiamin/non-fat calorie ratio           | . 83                      | 3        | 3.6               |
| B <sub>1</sub> /calorie ratio (Cowgill) | 65                        | 1        | 1.5               |

B<sub>1</sub>/calorie ratio is a better index and to a large degree independent of weight. They consider the following borderline values for the different ratios:

| Thiamin/calorie                 | 0.230 - 0.279 |
|---------------------------------|---------------|
| Thiamin/carbohydrate calorie    | 1.21 - 2.50   |
| Thiamin/non-fat calorie         | 0.251 - 0.300 |
| Vitamin B <sub>1</sub> /calorie | 1.7 - 2.29    |

Jolliffe states that at present he has insufficient data to express an opinion on this point but that in his clinic with the few cases observed he found greater urinary elimination of B<sub>1</sub> in smaller than in large individuals and that when both sizes were maintained on identical diets with a B<sub>1</sub>/calorie

ratio of 1.0, the larger individuals developed polyneuritis earlier.

Regardless of controversial points there is today no question that increase in calorie intake increases B<sub>1</sub> needs.

## FACTORS THAT INCREASE VITAMIN B1 NEEDS

It will be obvious from the preceding discussion that any factors that increase carbohydrate metabolism will involve vitamin B<sub>1</sub>. Jolliffe has listed some of these factors which are given in table 21.

#### STABILITY OF THIAMIN

We have seen that certain chemicals such as sulfites, nitrites and acetates can split the thiamin molecule into inactive fragments and that high acidity tends to protect against such disintegration. The loss of vitamin B<sub>1</sub> potency reported by Morgan to follow sulfuring of fruits may be due to such action.

It was the discovery that the antineuritic substance is heat labile that originated the fractionation of the vitamin B complex. According to Williams the effect of heat on thiamin is serious only in neutral or in alkaline solutions and probably merely aggravates the splitting tendency. He states that solutions of pure thiamin chloride hydrochloride may be sterilized by heating at 120°C. for half an hour or more and that this stability is probably due to the acidity of the salt which gives solutions of approximately pH 3.5. In the dry form thiamin is quite stable to heat and heating for 24 hours in contact with air at 100°C. produces no loss of potency in the crystals.

On the other hand, Elvehjem has shown that in cooking of meats there are relatively large losses of vitamin B<sub>1</sub> and cites the data given in table 22. Gunderson also reports that rolled oats boiled 5 minutes lose 12 per cent of their B<sub>1</sub> potency.

It is evident that generalizations as to the effect of heat on vitamin B<sub>1</sub> potency are impossible and that the effect of cook-

ing operations on particular foodstuffs awaits actual assay determinations such as those cited above. Guha and Drummond for example, showed that boiling a concentrated solu-

# TABLE 21 Factors that Increase Vitamin B<sub>1</sub> Needs (After Jolliffe)

#### I. Increase in Total Metabolism

- A. Abnormal activity, as associated with:
  - 1. Prolonged continuous activity
  - 2. Delirium
  - 3. Manic depressive psychosis, manic type
- B. Fever, especially of long duration, as in
  - 1. Tuberculosis
  - 2. Typhoid
  - 3. Malaria
- C. Hyperthyroidism
- D. Pregnancy
- E. Rapid growth

#### II. Faulty Assimilation

- A. Diarrhea, especially of long duration, as in
  - 1. Ulcerative and mucous colitis
  - 2. Intestinal parasites
  - 3. Intestinal tuberculosis
  - 4. Sprue
- B. Gastro-intestinal fistulae
- C. Diseases of gall bladder and liver
- D. Achlorhydria
- E. Carcinoma of the stomach

#### III. Increased Excretion

- A. Polyuria, as in
  - 1. Uncontrolled diabetes mellitus
  - 2. Diabetes insipidus
  - 3. Long-continued excessive fluid intake, as in urinary tract infections
  - 4. Lactation

tion of B<sub>1</sub> for 24 hours at pH 1 caused no appreciable destruction; at pH 5 resulted in 50 per cent destruction; and that at pH 9, one hour's boiling produced 50 per cent destruction.

The use of soda in the cooking of vegetables is therefore a menace to their vitamin B<sub>1</sub> content.

Being readily soluble in water there is danger of loss of vitamin B<sub>1</sub> potency through leaching out of the vitamin and when foods are cooked in water there may be appreciable amounts of the vitamin extracted in the cooking water, another reason for its conservation.

It has been noted that oxidation can convert thiamin to the physiologically inactive thiochrome. To what extent this occurs in cooking operations is totally unknown at present.

TABLE 22 (After Elvehjem)

| METHOD OF COOKING         | PER CENT<br>DESTRUCTION B1 |
|---------------------------|----------------------------|
| Beef round roasted        | 61                         |
| Beef round broiled        | 50                         |
| Veal quarter fried        | 45                         |
| Veal quarter roasted      | 58                         |
| Pork loin fried           |                            |
| Pork loin roasted         | 50                         |
| Pork ham fried            | 0                          |
| Pork ham smoked           | 10                         |
| Beef heart stewed 1 hour  | 55                         |
| Beef kidney stewed 1 hour | 40                         |

#### OTHER PROPERTIES OF THIAMIN

Thiamin can be made to combine with diazotized paraamino-acetophenone. Prebluda and McCollum have made use of this reaction and the red dye formed, to develop a colorimetric assay method for vitamin B<sub>1</sub>; a method that Melnick and Field have reported quite successful.

Thiamin has also been found to stimulate the fermentative action of yeast and Schultz, Atkin and Frey have developed this reaction into a quantitative assay method.

Thiamin has another property which proved of great value in isolation procedures, viz, ability to be adsorbed on the sur-



PLATE IX. Vitamins and plant growth. Various vitamins are quite as important to plants as to animals. This is well illustrated by these photographs which show the effect of thiamin and its intermediates on the growth of excised tomato roots cultivated in a solution of mineral salts and brown sugar. The upper photograph shows the effect of thiamin which has caused an eightyfold increase in growth. The lower photograph is of a similar experiment in which the media was enriched (reading from left to right) with thiazole, thiazole and pyrimidine and with thiamin. In this case the intermediates are as satisfactory as thiamin itself. Various plants differ in their requirements and powers of synthesis and this may be used in identifying certain vitamins and in assay. (Reproduced by permission of Dr. W. J. Robbins and The Botanical Gazette.)



face of certain adsorbents such as special forms of charcoal, fullers earth, frankonite, zeolite, etc. Seidell elaborated this procedure to the successful concentration of the substance from brewers yeast to a concentrate 100 times the potency of the original yeast. Such adsorption constituted the first step in the extraction of B<sub>1</sub> from watery extracts in the procedures of Jansen, Peters, Williams, and others. This property accounts for losses of vitamin B<sub>1</sub> potency in products that undergo filtration in their preparation such as beer. It must be reckoned with in conserving B<sub>1</sub> values whenever a vitamin B<sub>1</sub> source preparation involves filtering operations.

Winterstein, Williams and Ruehle found absorption bands at 235 and 267 m  $\mu$  respectively, whether the solvent was water or alcohol.

The commercial synthetic vitamin B<sub>1</sub> or thiamin chloride hydrochloride contains approximately one molecule of water of crystallization. It melts at 245°C. It is very soluble in water and slightly soluble in alcohol. Its aqueous solution, as already stated, shows a pH of 3.5 and is optically inactive. Narasimhamarthy reported that crystalline vitamin B<sub>1</sub> had an isoelectric point between pH 9 and 10.

#### VITAMIN B<sub>1</sub> POTENCY

There have been several ways of assaying vitamin B<sub>1</sub> potency and different ways of expressing such values. For that reason one needs a comparison table of equivalents to translate the studies by one method into the terms of another.

The present International Unit of vitamin B<sub>1</sub> is the equivalent of 0.003 mgm. or 3 micrograms of thiamin chloride hydrochloride. R. R. Williams has given a table of comparisons in table 23.

In this country many medicinal sources of B<sub>1</sub> are at present labelled in Chase-Sherman units. As shown above, International unitage is obtained by dividing the Chase-Sherman unitage by 2.

#### TOXICITY OF B1

Vorhaus, Williams and Waterman reported oral administration to man of a dose as high as 90 mgm. without the slightest untoward effect. Weiss and Wilkins used doses of 120 mgm. (40,000 I.U.) with no toxic effect.

More recently, however, certain evidence has been advanced to suggest that large doses of vitamin B<sub>1</sub> may increase fat deposits in the liver and that this tendency is counteracted to a degree in the presence of choline (Best and Ridout).

## TABLE 23 (After R. R. Williams)

1 gram of vitamin B<sub>1</sub> crystals assayed by a modification of the Smith ratcurative technique supplies:

300,000 International units

600,000 Sherman-Chase units

150,000 Smith curative units

300,000 Chick and Roscoe units

One International unit is equivalent to:

0.5 Smith curative units

2.0 Chase-Sherman units

1.0 Chick-Roscoe unit

20.0 Cowgill milligram equivalents

3.33 micrograms of crystalline B<sub>1</sub>

For this reason choline, as complementary to B<sub>1</sub>, may be given place in the vitamin group.

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## CHAPTER VIII

## BERIBERI

#### THE NATURE OF THE DISEASE

Beriberi, one of the commonest of the deficiency diseases, is of particular interest because study of it led to recognition of the entire group of avitaminoses. It existed, and still exists, chiefly in the Far East where it is a major cause of ill health. The name is believed to indicate that the gait of beriberi patients is like that of sheep. The Japanese term, kakke, also means a disease of the legs. But our interest in beriberi has been kept alive not by the classical disease as it occurs elsewhere but by the accumulating evidence that certain forms of it are common among our own people.

It has seemed wise to describe beriberi in considerable detail, because by chinical considerations beriberi, as it is known in Japan, seems to be a remarkably pure form of thiamin deficiency disease. At any rate none of the stigmata of other deficiency diseases have been present during the major outbreaks of beriberi. Thus the general nutrition appears to have been good, no mouth, tongue or gastro intestinal lesions or disturbances have been noted, no skin lesions suggesting pellagra or vitamin A deficiency, not even changes in the blood. Under these circumstances the excellent and considerable Japanese literature on the subject might be presumed to paint a clear picture of thiamin deficiency in man which should be useful in the further discussion of thiamin deficiency which we see in our own country.

The incidence of beriberi is largely a matter of speculation because it is most common in countries where there are either no statistics or very sketchy records. In 1934 Cowgill assem-

bled what data were available and the following brief account is drawn mainly from his monograph.

The incidence is highest in China, Japan, Brazil, the Philippine Islands, and the Malay Peninsula. In Japan the death rate in 1929 was 9.9 per 1000 deaths according to the International Health Year Book. It is the eleventh most common cause of death of infants in Japan. Grey found that 80 out of 500 women employed in the Central Telephone Exchange were incapacitated by mild beriberi and that 82,000 out of 208,000 cases of sickness in the Japanese Army in 1923 were cases of nutritional disease, largely beriberi.

Between 1891 and 1910 there were annually about 10,000 cases in Hong Kong out of a population estimated at 350,000 and in the same period 17.3 per cent of all hospital admissions in the Malay Peninsula were cases of beriberi.

As recently as 1918 beriberi was the third most common cause of death among infants in the Philippines. This is especially illuminating because, until 1904, the infantile beriberi was not recognized in the Islands, having masqueraded under the name of "taou" or "Suba" disease. Subsequent developments emphasize the difficulty in determining the incidence of nutritional diseases where they are not familiarly recognized as such.

Among the poorer classes the disease is still common in Japan. Thus Yanagi, in 1937, reported 14.2 per cent of 888 clinic patients afflicted, approximately the same incidence as had existed there for ten years. One out of every ten hospital admissions was a case of beriberi.

The conditions associated with thiamin deficiency in our own country are also very common although they less frequently reach the advanced stages of beriberi. But such speculation is worthless since the implications extend far beyond the clinical manifestations of deficiency. Thiamin deficiency is certainly a major cause of disease and physical infirmity.

# THE MODERN HISTORY AND ETIOLOGY OF BERIBERI

Beriberi was considered due to diet long before the conception of deficiency disease. It was produced experimentally by Forster in 1873 but incorrectly ascribed to mineral deficiency. The modern history of the disease commences with Eijkman and Grijns' investigations and their recognition of the part played by the rice pericarp in preventing experimental beriberi. The next important step in proving the nature of the disease was taken by Frazer and Stanton who took 300 Javanese laborers into the jungle. Half were given polished rice, half less refined rice as the major item in their diets. Beriberi developed in the first group after three months whereupon the groups were reversed and the second group became sick. In other words, having controlled environmental factors and under sanitary surroundings the seemingly insignificant change in food associated with feeding polished rice was found capable of producing or preventing beriberi. Four years later similar results were secured in Manila by Strong and Crowell.

The disease has frequently been produced in volunteers since that time. Shimazono describes an 18 year old male who was given a diet poor in thiamin. 31 days later his appetite became poor, the following day he vomited. On the 41st day hyperaesthetic areas were present on his legs and this was followed by slight edema. His ankle jerks disappeared on the 49th day and his condition thereafter became rapidly worse. On the 70th day his condition was considered dangerous and he was given a large dose of rice polishings concentrate. Recovery was prompt. Appetite became normal within 2 weeks. However, sensory disturbances persisted for 5 months and motor changes could be demonstrated until the 8th month. Essentially the same events were observed by Bröder and Engel in a young German girl who had starved herself in order to reduce. All of the classical symptoms of beriberi appeared and were cured by thiamin and diet.

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Experiences of this kind and the uniform success experienced in producing the experimental disease at will quickly led to the belief that the deficiency theory was correct. However, many continued to believe in the infectious nature of the disease. In 1913 the Far Eastern Association of Tropical Medicine united in declaring beriberi a deficiency disease. Ten years later this was modified somewhat and the Association could only agree that it was nutritional. One cause of this difficulty was that in various animals the experimental disease differs from the spontaneous one. Thus the experimental animal usually is emaciated before nervous symptoms appear, which is not true of human cases. But more important is the absence of cardiac hypertrophy in experimental beriberi (in rats and fowl). Moreover the rat shows adrenal hypertrophy, which the human case seldom does, and does not show edema which most human cases do. In the monkey and the dog the experimental disease is more nearly the counterpart of the human one. Even in the rat more similarities than differences may be found. At the present time the proof is overwhelming that deficiency of thiamin is the cause of the disease and that the differences are due to the nature of the different species of animals used.

The steps by which thiamin deficiency induces the symptoms of the disease are not as clear. Presumably one role is in the disposal of pyruvic acid. This is indicated by the experimental studies of Peters which have been discussed in an earlier chapter and the discovery of accumulations of pyruvic acid in the blood of human cases of beriberi. It has also been suggested that methyl glyoxal, a pyruvic aldehyde, rather than pyruvic acid is responsible. Williams has called attention to the extensive experience of Japanese physicians which emphasizes the importance of methyl glyoxal-like substances and the toxic character of the milk from women suffering from beriberi. Like substances have been demonstrated in the blood, urine and tissues of experimental cases

of the disease. Such an explanation of the pathogenesis of beriberi, i.e. deficiency operating through a toxic substance, would explain many of the features of the disease.

# THE MORBID ANATOMY OF BERIBERI

The clinical differences between acute and chronic cases of beriberi have their counterpart in the appearance of the subjects on the autopsy table. The acute cases are often in good fat and show less degeneration of the nervous tissue than the chronic cases but the appearance of the heart is more striking since death is usually due to a cardiac attack. On the other hand the chronic cases seldom die unless complications intervene, and in such cases the appearance of the various organs is more the result of the tuberculosis, typhoid fever, or other infectious disease than of the beriberi itself.

The anatomic distribution of the nervous lesions conforms with the clinical manifestations. The nerves supplying the lower extremities are most commonly affected but the cranial nerves and the vagus system are frequently deteriorated too. The peripheral portions of the nerves are first and most seriously altered. The cranial nerves above the 7th are rarely involved.

The nervous lesions are not recognizable macroscopically. Histologically they consist of a pan-neuritis which commences with vacuolar degeneration of the cells of Schwann. Later, fragmentation of the axis cylinders occurs and Wallerian degeneration may be demonstrated. Fat stains are suitable for studying the fatty degeneration. Collections of round cells appear in the nerve bundles.

The central nervous system is but slightly affected. The membranes of the brain are sometimes thickened and the ganglion cells in pons, medulla, and spinal cord are found degenerated. Since such changes are extremely common in adults and may result from a wide variety of morbid processes they are of little or no value in arriving at a diagnosis. The

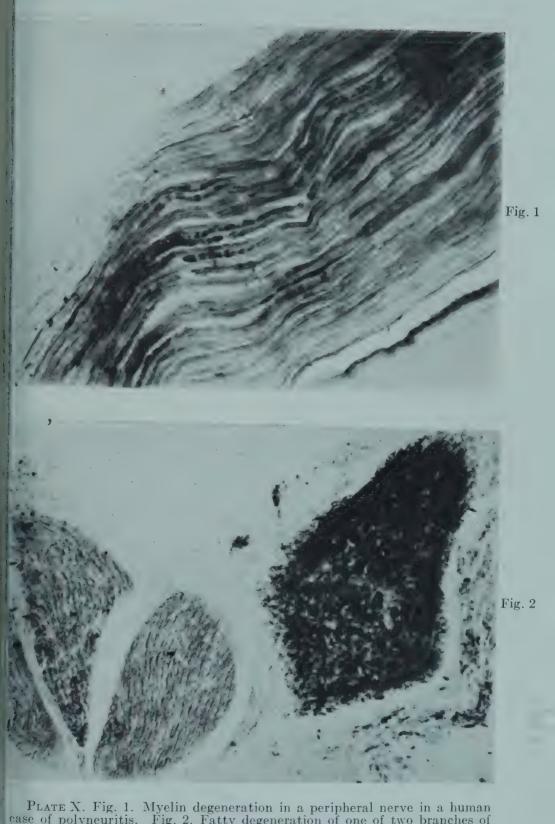


PLATE X. Fig. 1. Myelin degeneration in a peripheral nerve in a human case of polyneuritis. Fig. 2. Fatty degeneration of one of two branches of the vagus nerve in a woman whose death was associated with great dilatation of the right chambers of the heart. The right nerve is affected. No other organic changes found. The case is believed to have been one of beriberi. (Stained with Oil Red O.)



ganglion cells show swelling of the cell bodies, peripheral displacement of the nuclei and partial or diffuse disappearance of the Nissl substance. Occasional fibers in the cord and brain show fatty degeneration. The cerebrum is otherwise unaffected.

Degenerative lesions are common in the muscles of foot and leg, although in some cases similar changes occur in the upper extremity, thighs, and diaphragm. The lesions are not characteristic histologically, consisting only of cloudy swelling, fatty degeneration, loss of striations and hyalin or waxy degeneration. In "wet" cases edema will be recognized in the muscle preparations and in the chronic, wasted cases an atrophy of the muscle fibers.

### Heart

The acute cases reveal more illuminating changes in that the heart is involved. The most characteristic effect of the disease on this organ is its appearance in situ. Wenckebach fixed the hearts in his cases with formaldehyde solution before removing them, to preserve the shape and relative sizes of the various chambers.

Older observers have almost uniformly ascribed the cardiac manifestations to lesions of the vagus nerves. The only evidence for such an explanation has been the frequently described vagal lesions. But there is no evidence that vagal lesions ever produce the changes seen in beriberi, and good cause to doubt that vagal degeneration and cardiac symptoms go hand in hand. Indeed, the heart symptoms are seen in acute cases when the nerve lesions are scanty or underdeveloped.

The organ is hypertrophic and dilated. The average weight of the heart among Japanese patients has been given as 368 grams, while the maximum of normal hearts among the same population is 300 grams. Cardiac enlargement has been measured in 200 cases by Kobayashi using x-ray measurements. He found that the lung-heart quotient of Groedel is reduced

from a normal value of 1.71 to between 1.19 to 1.43 in beriberi. The method was recommended as an aid in diagnosis. The enlargement of the heart is most pronounced in the right chambers and the left heart may be very small. The right auricle is huge, with paper thin wall through which the dark blood within may be seen. The walls are said to be friable and easily torn. The conus arteriosis is tremendously dilated, especially at the point of origin of the pulmonary artery. Wenckebach considers this to be pathognomic of this form of right sided dilatation. There may be petechiae at the auricular-ventricular juncture. The lungs and the left heart are not over-filled with blood. The impression is that the right chambers are so feeble that they steadily enlarge as the site of least resistance. Epicardial petechiae and flecks of fibrin on the epicardium have been reported. Shimazono mentions fatty degeneration in the bundle of His'. Weiss and Wilkins found hydropic degeneration of the muscle fibers, swollen collagen and perivascular edema and separation of the muscle bundles. These lesions were not constant.

Sambuc examined 52 cases of beriberi in Cochin-China and considered the appearance of the heart pathognomonic. The degree of dilatation, the location of the dilatation in the right side and the pericardial effusion constituted an unmistakable picture. Sambuc ascribed death in some cases to pressure from the effusion. Other cases died from rupture of the auricle.

The gross criteria of beriberi used by McLaughlin and Andrews are: (1) Dilatation and hypertrophy of the right heart; (2) congestion of the viscera; (3) anasarca, and (4) absence of other findings to account for death.

No satisfactory anatomical explanation of the heart lesions in beriberi exists. Microscopic examination shows nothing of importance. To circumvent the impossibility of examining heart muscle during the acute phases of the disease Wenckebach took small specimens of the calf muscles which he felt might be

comparably affected. They appear normal in all respects but size. In the acute stage the fibers were larger than normal by actual measurement, but well marked and of usual structure. A second sample was taken from the same subject after treatment and showed the muscle fibers to be of normal dimensions. Wenckebach considers that the striated muscle of the calf had excessive bound fluid within its fibers and, by analogy, the heart muscle also. More recently Porto has described interfibrillar and interfascicular edema and perinuclear vacuolization in the muscle and conduction system cells of the hearts of dogs suffering from thiamin deficiency. He also observed some alterations in the appearance of the muscle striations and gross changes similar to those just mentioned as characteristic of human beriberi.

Anasarca, hydrothorax, and hydropericardium are very common in acute cases of beriberi, being seen also in some chronic cases. The presence of transudate in these cavities, as well as the degree of dilatation of the heart depend in large measure on the condition of the patient before death. Persons who have been active are more liable to show such changes, while bed rest permits compensation and permits the heart to return to more nearly normal dimensions, and for fluid to be resorbed.

On the basis of such observations the fluid in "wet" cases of beriberi has been considered to be purely a transudate. The edema of the lungs and liver (in which cases congestion and central necrosis in the latter also occur) have been interpreted similarly. However most cases of beriberi have been judged by rather primitive criteria and it is not surprising to find that recent studies have denied the transudate theory. Wenckebach's views imply another mechanism. Eppinger has commented on Wenckebach's material and given his interpretation of the lesions in the liver specimens collected by Wenckebach. He considers the fluid to be the product of a serous inflammation and found histological lesions of serous

hepatitis in all cases. These changes included the widening of Disse's spaces (the controversial lymphatics said to lie between the capillaries and liver cells), lymphatic stasis and periportal edema. Edema was also present in the bed of the gall bladder. Toll has found a thickened gall bladder wall constantly present in beriberi.

It should be apparent that this view, implying the presence of an irritant, is much more in harmony with the work of Peters than the older conception and that while it still lacks the support of other investigators offers some hope that further advances in the clarification of the pathogenesis of beriberi may be expected from anatomic studies.

Eppinger's views are not incompatible with the observation previously noted that active cases present more free fluid in their tissues and serous cavities. The relationship between vitamin B<sub>1</sub> requirement and metabolic rate suggests the deficiency may be more aggravated in active patients and the production of irritants due to faulty metabolism increased.

The pancreas is at times shrunken and even slightly cirrhotic. The islands of Langerhans are either not affected or are hypertrophic in conformity with the altered carbohydrate metabolism seen in beriberi.

The adrenals are sometimes enlarged due to medullary hypertrophy. A slight lymphocytic infiltration is frequently mentioned. Nagayo and Katsunuma reported hypertrophy of the hypophysis and thyroid glands and Katsunuma found lymphocytic and plasma cell infiltration in the pars intermedia of the hypophysis in all his cases.

The lymphatic apparatus is not reduced in acute cases. Atrophy of these tissues is generally believed due to inanition and not beriberi. Nagayo, indeed, found cases with status thymico-lymphaticus and considered what part this may have played in their sudden exodus.

The cervical and splanchnic ganglia and plexus of Auerbach have all been reported as showing degenerative changes. The larger portions of the vagus have all been found showing changes similar to those in other nerves. Changes have been reported by many pathologists in the nerve endings, both motor and sensory but these are difficult to study. In infants, Honda found lesions of the laryngeal nerve but not of those in the extremities. This is probably explainable on the basis of usage.

Vedder has written that "although the symptoms of beriberi have been attributed in the past chiefly to peripheral neuritis, the condition is not a simple polyneuritis but is a degeneration of the entire nervous system." This opinion is based in part on the finding of lesions in the cord, nerve roots and ganglion cells of the medulla and pons. It is probably a sound point of view. Certainly the lesions of the nervous system have been overemphasized in beriberi. And surely, too, they are scant help in the recognition of thiamin deficiency. Broder has recently written on this subject and pointed out how poor a criterion of various deficiency states the neuropathy is. Kihn and Davidson's studies also emphasize this point of view.

It may be seen from the above that the post mortem studies of beriberi have contributed little as yet to our understanding of the pathogenesis of the disease. The diagnosis of beriberi post mortem must depend on the association of a rather characteristic cardiac dilatation and hypertrophy without evidence of organic cause and degeneration of the peripheral nerves or, in the chronic cases, a disseminated, irregularly distributed myelin degeneration with or without wasting or edema. To these rather unsatisfactory criteria may be added other of the lesions described.

It is to be ardently hoped that future studies will sharpen our perception of the essential changes in this interesting disease to such a degree that we can identify not only the well developed case but those incomplete forms which now pass unrecognized.

## EXPERIMENTAL BERIBERI

Eijkman's experiments were based on fowl fed only polished rice. In the third week of such a diet the animals become paralyzed and the head is retracted. Within a few days they die unless thiamin is given in which case complete recovery occurs within a few hours. Two things stand out from this story. The diet is obviously deficient in many elements besides thiamin and the duration is much too brief to permit the development of organic changes such as characterize beriberi. McCarrison recognized this artificiality of fowl polyneuritis a dozen years ago and modified the diets by supplying small amounts of vitamin. His animals thereupon developed lesions and behavior more like the natural disease, including enlargement of the heart.

The same thing occurs in rats. The usual laboratory diet, composed of casein, starch, salt mixture, cod liver oil and butter fat and supplemented with the other B fractions produces marked loss of appetite within 3 weeks and later nervous disturbances. The hind legs became spastic and stiff and the animals often move in a circle. When held by the tail the rats revolve in a characteristic "circus movement." These effects are due more to inanition than thiamin deficiency and the anatomical changes bear this out. True paralysis can only be produced by feeding a thiamin low rather than completely deficient diet.

## Lesions of the Nerves

A recent study by Prickett, Salmon and Schrader, in which the discrepancies of earlier workers is reviewed, seems to demonstrate that under comparable conditions lesions in the nervous system may be produced in rats identical to those which occur in man. The examinations were made largely with Nicol prisms. "At autopsy the peripheral nerves of a few animals that had shown severe symptoms were observed to have localized enlargements along their course." histologic changes included swelling of the sheath and axis The nodes of Ranvier were enlarged and dumb-bell In the severe cases considerable isotropic material was present and the axis cylinders roughened and irregular. Yet the occurrence and severity of the lesions was to a considerable degree unpredictable as other workers had found. greatest success in correlating nervous lesions with clinical behavior appears in Swank's study of thiamin deficiency in pigeons. Swank found opisthotonus an expression of acute deficiency or a terminal manifestation in partially deficient birds. In the former case it was not associated with nerve lesions. In the partially deficient animals however nerve lesions were quite uniform and were constant if weakness of the legs had been noted clinically. The number of degenerate fibers in the sciatic nerve corresponded to the degree of paralysis. Regeneration was followed in treated animals.

In conformity with earlier reports Swank found the longest nerves to degenerate first and degeneration to commence in the distant parts of the fiber. Degeneration was associated with chromatolysis and eccentrically located nuclei in the dorsal ganglia. In the spinal cord degeneration was observed mainly in the ventral funiculus and spino-cerebellar tracts. Swank refers to unpublished observations which indicate that starvation induces only sheath changes (fatty droplets) and that evidence of nerve fiber deterioration is essential to a diagnosis of thiamin deficiency. His report is also interesting in that many of his pigeons died with evidence of congestive heart failure (congestion of the lungs and liver) and some of these showed focal necroses of the myocardium. Hydropic degeneration was never seen.

No mention is made in most reports of the condition of the terminal portions of the nerves. Tsunoda and Kura, however, describe changes in the ends of both sensory and motor nerves which were observed to respond within 5 hours to specific therapy. Woolard reported similar lesions. Woolard described the lesions in the motor terminae as swelling with bulbous enlargements and loss of detail. These were the first manifestations of deficiency and were followed by changes in (a) the sheaths and (b) the axis-cylinders.

Completeness requires some mention of the view that the nerve lesions in beriberi, both experimental and human, are not due to thiamin deficiency at all. This is a perennial theory recently restated by Engel and Phillips who based their opinion on experiments with chicks in which all dietary factors but thiamin were supplied and in which no lesions were encountered. Lipschitz, Potter and Elvehjem have also examined the issue and suggested that polyneuritis is primarily a disease of disturbed carbohydrate metabolism rather than of lesions of the nervous system. With the latter view it is impossible to take exception. The question is whether the functional pathology, doubtless of a very special and specific type, does not produce equally constant structural changes. The perfection of anatomical techniques and understanding will very likely reveal that such is the case. In the meantime the observations which have been made show that the nervous system is the logical place to look for them. It is possible that the lesions, and the symptoms, are due to the accumulation of pyruvic acid or some other product of disturbed metabolism. However the symptoms of beriberi have not been induced by the injection of pyruvate and there is not a close correlation between bisulfite-binding substance in the blood and the severity of symptoms. Williams and Spies believe that judgment on this point should be reserved since the amounts injected have not been large enough to exclude this possibility. Other dietary factors, especially other members of the vitamin B complex will need to be very carefully controlled before the ramifications of the subject of nervous lesions can be laid bare but this becomes steadily more easy.



PLATE XI. Gastric ulcers in rats fed a diet deficient in vitamin B<sub>1</sub>. Figure 1 shows a typical acute erosion and figure 2 a chronic peptic ulcer similar to that seen in man with extensive vascular lesions and scar formation. (Reprinted by permission of the Journal of Experimental Medicine.)



# Gastro-intestinal System

There has been a steady growth in our knowledge of the effects of thiamin deficiency on the gastro-intestinal system. The functional disturbances are well recognized. Loss of appetite was firmly established by the work of Cowgill, Deuel, Plummer and Messer demonstrating gastric atony by means of a balloon inserted through a gastric fistula. Cowgill and Gilman showed that gastric acid (in dogs) was reduced or absent. Atony of the intestinal tract as well as the stomach was demonstrated by Rowlands many years ago and degeneration of Auerbachs plexus was reported by McCarrison. Sparks and Collins more recently have re-examined the atony effect of thiamin deficiency. Their method was to actually measure the capacity of the large bowel. The bowel was first cleansed and then filled with a radiographically opaque fluid to the ileocaecal valve. In the normal rat this required from 3 to 4.4 cc. Using rats on a thiamin deficient diet the capacity was greatly increased in 70 per cent of the animals. In most of these the capacity was doubled. The effect was believed due to thiamin alone.

Gastric erosions and ulcers occur in thiamin deficient rats. McCarrison first observed this. In the experiments of Dalldorf and Kellogg the incidence was high and the chronicity of the ulcers seemed related to the duration of the deficiency. Partial deficiency was necessary, the rats being maintained near the level of symptoms for some months. Recently Thatcher, Sure and Lee, in a comprehensive study of the histopathology of the avitaminoses state: "Perhaps the most significant result in the investigation is the finding of gastric ulcers as a result of the specific influence of a deficiency of vitamin B<sub>1</sub>." Inanition was controlled by holding the controls to the same intake as the experimental animals. Other B factors were supplied. Of 8 rats 5 had ulcers.

Simpson failed to find ulcers. Adult animals were used. The experiments extended over 4 months. Ulcers have been

found by Schiödt, Drummond et al. and Howes and Vibier. The latter workers report that young animals are more prone to develop ulcerations than adult ones which may explain Simpson's failure. Drummond's experiments are very conclusive. More than 1000 rats were used, the deficiency was very slight but prolonged throughout the life of the animals. Ulcers and erosions were common in the deficient animals. Most of these lesions are simple, punched out erosions. However, erosions are believed to be the first stage in the formation of chronic peptic ulcer and in Dalldorf and Kellogg's rats chronic, indurated lesions were occasionally found. We have found no record of gastric ulcers in human cases of thiamin deficiency. The mechanism by which they occur in the rat is obscure. Mid-brain lesions are often associated with gastric ulceration in man and the dog and the "chemical lesion" in experimental beriberi involves this part of the brain. production of ulcer by vagotomy, recently reviewed and studied by Beazell and Ivy, may afford a clue. Considerable evidence also exists to show that the terminal nerve structures in the gastric wall are diseased in peptic ulcer. Stöhr's studies have confirmed this and shown that these minute lesions are widely distributed in the gastric wall. The cells of both Auerbach's and Meissner's plexi are altered, an observation made in experimental thiamin deficiency by McCarrison.

A degree of deafness was demonstrated in rats and chicks on a thiamin deficient diet by Selfridge and Maurer and Tsai, by means of maze tests, showed that partial depletion stigmatized the learning ability of their animals. In the latter experiments the deficiency was multiple. It would appear that the entire nervous system suffers from lack of thiamin and that the occurrence of lesions is determined by secondary and often local factors. This is in harmony with the lesions in man to which reference has already been made.

# Glands of Internal Secretion

The adrenal glands are hypertrophic in experimental thiamin deficiency and Ogata found lesions in the associated ganglia as well. During the active phase of the deficiency the cortical cells show considerable mitotic division. The islets of Langerhans are also hypertrophic (Wolbach, Ogata, Ueno). Kihn found this difference small but constant in rats while in pigeons and fowl the hypertrophy more than doubled the normal weight. The thymus, pituitary, thyroid, spleen and liver are reported to be atrophic. Whether this is due to inanition is unknown. Earlier reports of atrophy of the seminiferous tubules and disappearance of sperm cells are believed by Evans and Bishop to be due to other deficiencies. However, loss of libido occurs in male animals and prompt suppression of follicular function in the ovaries of females. Evans and Bishop showed this to be an early effect of the deficiency. The phenomenon was extensively studied by Ueno who was able to maintain normal estrus by administering follicular and anterior pituitary hormones.

#### SYMPTOMS OF EPIDEMIC BERIBERI

It is customary to consider the nervous signs of beriberi the first to appear. This is probably not true. Significantly, Shimazono, in characterizing the disease, lists the three major features in the following order: cardiovascular symptoms, edema, and neuritis. The usual sequence is mild cardiac symptoms with slight edema and dyspepsia followed by nervous symptoms.

# Circulatory Manifestations

In the early stages of the disease palpitation is present and dyspnea occurs with every effort. The heart action is increased, the apex extends beyond the mid-line and epigastric pulsation is very common. The outline of the heart is enlarged

and rounded. Miura states that enlargement is first to the right, then to the left and upwards. During recovery the right margin is the first to return to normal. The heart sounds are exaggerated, especially the second pulmonic one and the pulse is rapid. In severe cases the rate exceeds 100 p.m. The rate is very labile and responds to the slightest exertion. The pulse wave is large and full. A rate of 120 to 130 in acute cases, or chronic ones which have become acute, is a warning signal. The rate slows rapidly under treatment and may become abnormally slow during convalescence. Systolic pressure is not affected but the diastolic pressure is almost constantly low. A murmur may be heard over the veins, particularly the femoral one. Miura heard this cruraltone when standing at the bedside of a patient.

Wenckebach described in detail the cardiac symptoms as he saw them in Java. He felt the following to be particularly helpful in establishing a diagnosis:

- 1. Enlargement of the heart by percussion, auscultation and x-ray examination.
- 2. The presence of murmurs, chiefly systolic but also presystolic, with a resonant first sound. The murmurs are disproportionately increased by exercise.
- 3. Visible and palpable throbbing pulsations over the heart, best felt just to the left of the sternum.
- 4. Bounding pulse and thrill over the great arteries.
- 5. Over-distended neck and arm veins and, without exception, a painful swollen liver. In the most severe cases liver pulsation.
- 6. The electro-cardiogram remains normal throughout, though a slight shortening of the auricular interval has been reported.

## Nervous Symptoms

The neuropathy of epidemic beriberi is strictly peripheral The psyche remains clear. We have found no record of psychosis of the Korsakoff type in the Japanese literature From the beginning of the disease the patients complain of disturbed sensation in feet and legs, sometimes in finger tip BERIBERI 177

This disturbed sensation is described as though their skin was covered with a sheet of paper. Later pain and weakness occur in the legs, walking is tiring and cramps may occur in the thighs or calves at night.

The sensory disturbances begin in the feet and legs but both the finger tips and a zone about the mouth may be affected. Miura states that a fourth area of hyperaesthesia is the skin of the lower abdomen below the umbilicus. The areas of hyperaesthesia do not correspond to the distribution of particular nerves and their margins are indefinite. The sole of the foot is much less affected than the dorsal surface and the perineum is very rarely involved. However in severe, chronic cases the forehead and skin about the eyes may be affected. In these cases the sensory changes in the lower extremities show considerable anaesthesia to touch, not to pain. As the sensory changes develop epicritic sensation is first affected, then temperature and pain sense. Vibratory sensation is also disturbed. It is said to be the last to return with recovery.

Position sense is destroyed to a slight degree.

The motor disturbances also begin in the legs and usually are limited to the lower extremities. All degrees of change from weakness to paralysis have been noted. Toe drop is present with motor lesions; the gait becomes broad based. In such severe cases the hands may be weak and Miura described a peculiar position which the hand assumes in which it hangs limply with but slight flexion in the phalangeal and metacarpal-phalangeal joints. In these cases the diaphragm and recurrent nerve and sensory branch of the 5th cervical nerves are paralyzed. Head and neck movements are not limited. Laryngeal paralysis, edema and congestion quite frequently occur. Facial weakness is bilateral and consists of poor closure of the mouth, slight ptosis of eyelids and limited extension of the tongue. This is an uncommon sign. and Shattuck are of the opinion that the case of paralysis described by Landry and from which the term Landry's paralysis

has arisen was a case of beriberi. Shattuck feels that an important group of ascending paralyses are due to thiamin deficiency.

The motor disturbances, like the sensory ones, are frequently more severe on one side than the other. If both motor and sensory changes are unequally present they are almost invariably most severe on the same side. The reflexes in the lower extremity are first over-active and then gradually become weaker and weaker. The sphincters are not involved. Abdominal muscle weakness may cause difficulty in urination.

Little is found in the eyes. Certain writers have reported cases with night blindness and amblyopia due to central scotoma. Taste and hearing are unaffected.

Pain on pressure is commonly met with in the muscles of the calves early in the disease. This is an important diagnostic point. The nerves are not tender to pressure.

## Edema

It is said that experienced physicians, in countries where beriberi is common, often suspect the disease from the pallor of a patient and a slight degree of edema of the legs. The edema is not related to the cardiac function or to disturbances in the kidneys but probably is related to the vascular function. In the mildest cases it is precipitated by exertion, ir more advanced cases it becomes permanent. If the patient be bed ridden it occurs in the back and shoulders. It is often associated with slight pleural effusion but the latter may occur without edema. No satisfactory explanation has been of fered for the edema. It is present to some degree in mos cases but in only a few does it become prominent. The sug gestion has been made that it represents a protein deficiency but proof is lacking and clinical facts do not support thi view.

Schretzenmayr has made an interesting observation regarding the edema of beriberi. It is, he writes, never deform

ing. If the face is involved the features are not distorted as is true of nephritic edema.

Of the various forms of beriberi, the "wet" and "dry," the acute and chronic, more will be said later on. It is more important here to note that in beriberi countries it has been well recognized that a stage of incipient beriberi may occur, so mild the patient may hardly be called sick at all and that a latent form of the disease marked by residual signs is common after recovery. Thus, in examining a group of factory workers, Shimazono observed many with low diastolic blood pressure. More careful examination revealed mild beriberi in all of these men. The observation is all the more striking because such individuals, as indeed most patients with uncomplicated mild attacks, are in good fat and look to be well nourished. Indeed, most cases keep their weight during the attack, the exceptions being the acute cases with vomiting and the edematous cases which become "dry."

Fever occurs only in the acute form of beriberi and in these cases for but a few days before death. The fever in other cases is always due to another, coexisting disease.

The acute cases of beriberi may show a slight hyperglycemia, in other cases the sugar, calcium, albumin-globulin ratio are normal. The blood morphology is not affected. The anemia seen in some cases is explainable on other grounds, parasitism, etc. Shimazono believed that eosinophilia was a rather constant feature of the blood, probably due to nerve damage and Nakamura reported the platelets increased in number. The urine is not qualitatively changed but is suppressed in many cases. Sudden diuresis of large amounts of urine, two to four liters, is sometimes observed during recovery.

A sense of pressure or pain in the stomach is complained of by many patients as well as poor appetite and eructations. Scheube found gastric symptoms in 25 per cent of his cases. Most writers consider them commoner. They are a feature of the early stages of the disease. The acute form, Shoshin,

is characterized by vomiting, first of food, later of gastric secretions. Vomiting occurs as a result of the slightest movement or effort. Constipation is frequent, diarrheauncommon.

Kitamura and Shimazono followed the gastric secretion in many cases and found it reduced from the earliest stages of the disease and developing into achlorhydria. This process could be reversed by treatment. Goodhart and Sinclair studied 100 individuals and found a definite association between the

blood cocarboxylase and gastric acidity.

The vital capacity is reduced in beriberi. Weakness of the diaphragm and abdominal muscles is largely responsible Fukui demonstrated this by inserting a rubber balloon into the rectum of patients and measuring the changes in pressure No respiratory pulsation occurred in the advanced cases o beriberi. The severe cases of cardiac failure are complicated by pulmonary edema.

# Beriberi in Infancy

Beriberi in infancy is an interesting expression of the deficiency first recognized clinically by Hirota fifty years ago. It affects breast fed infants and only those whose mother have beriberi. Not all beriberi women have beriberi infants. In some cases the mother is but slightly affected by the diseas while the infant is severely afflicted. Shimazono states that those cases which he has investigated, in which the infant was sick and the mother appeared normal, all showed some physical signs of beriberi in the woman.

The cases occur in the summer and early fall, nearly twice as frequently in male as female babies and the peak incidence is in the second month of life. The cardiac signs are pronounced but the earliest and most important symptom is los of appetite and vomiting. Ohta found that these little patients had a small gastric capacity, could take but a small amount of milk at one time. Urine is scanty, face pallice

pulse and respiration labile and rapid. The cranial nerves are much more commonly affected than in adult cases.

Loss of voice—aphonia—is also common among infants and is said to be highly characteristic. The child can only moan or whine in a plaintive fashion and the "beriberi cry" has made so distinct an impression on clinicians that the diagnosis is often made on the basis of this one symptom. Eighty-five per cent of Ohta's patients were hoarse. Laryngoscopic examination showed paralysis of a vocal cord in each case, the left cord being more frequently involved than the right. Drooping of an eyelid—ptosis—was also common. Ohta observed that the slightest infections such as common colds resulted in exacerbations of the beriberi. Some physicians have attributed the laryngeal paralysis to pressure by the dilated right auricle.

Diagnosis is usually made only after the onset of acute symptoms, the most characteristic being sudden paroxysms of pain associated with rigidity of the body which is held tense and straight. No true convulsions occur. The face is cyanosed, the neck veins engorged, the pulse small and rapid. The pulse rate rapidly increases on movement, being very labile. The infant cries in a low plaintive voice and has repeated attacks of similar nature until death occurs, often within twelve to twenty-four hours, unless treatment is effective.

In forty-eight of Ohta's cases special studies were made of the gastro-intestinal function (Ohta and Izumita). The group as a whole showed reduced total acidity and free hydrochloric acid although the values in the individual cases were extremely variable. The daily fluctuation in acidity was abnormally pronounced and this fluctuation, as well as the amount of acid, returned to normal under the influence of vitamin therapy. However the response is not always prompt. The authors divided their cases into four groups, depending on the type of gastric dysfunction present. The majority of their cases fell into a group characterized by anacidity or

hypoacidity. In this group the reduced acidity was found to persist for from two to three months after the beginning of the treatment.

No delay in the passage of test meals was observed by these authors who found normal values for the emptying time of stomach, small bowel and colon.

Vedder has described an uncommon form of the disease in infants which runs a protracted course and is marked by obstinate constipation, vomiting, usually occurring every day at about the same time but unrelated to meals; restlessness at night, enlarged heart and developing pallor and weakness. Bray's experience seems to have been somewhat similar. He noted on Pleasant Island, in the Pacific, that coincidental with a change in the native diet, the substitution of canned foods for the native "toddy," the infant mortality increased alarmingly. Breast fed infants were most affected. The symptoms were vomiting, distension, irritability, intense cyanosis and convulsions. Death occurred suddenly. At necropsy the right heart was distended. Some of the infants had symptoms of meningitis or pneumonia but bacteriological examination was negative. The duration of the disease was very brief, 12 to 24 hours, and vitamin therapy successful. Brav described a different clinical syndrome in older children which he also described to thiamin deficiency. The symptoms were loss of weight and appetite, subnormal temperatures and susceptibility to infectious diseases such as bronchitis, otitis media and pharyngitis. The fatal cases also showed right sided heart failure and degenerative changes in the nerves.

# Ship Beriberi

Atypical forms of beriberi encountered in our own country are discussed later on but one type, ship beriberi, is best considered in connection with the epidemic disease since it is no longer a medical problem. The disease has not been well studied because it has occurred almost entirely on board

sailing ships which seldom carried a medical officer. The nervous symptoms of beriberi appear in characteristic manner as well as weakness, constipation and loss of appetite but other symptoms are common which are not the result of thiamin deficiency. Thus skin petechiae and hemorrhagic gingivitis are often reported which are due to scurvy and night blindness which is due to vitamin A deficiency. Protein deficiency edema has undoubtedly been present in some instances as well.

## Predisposing Factors

Six predisposing factors are listed in the Japanese literature:

- 1. Temperature. Cases are commonest in the warm months. This seems independent of fluctuations in the thiamin content of the food.
- 2. Humidity. Both the military and industrial records show that high humidity predisposes individuals to beriberi.
- 3. Age. It is a disease of young people, the majority of the patients being between 15 and 30 years of age.
  - 4. Sex. Males are affected 2 or 3 times as often as females.
- 5. Robustness. Active individuals seem to be predisposed to beriberi. Scheube noticed this in 1894. Army records show that forced marches precipitate attacks. Shimazono observed 8 cases among girls working in a spinning plant. They sat at their machines and worked almost entirely with their left hands. All 8 had hyperaesthesia of that part of the body and no other.
- 6. Physiological strain. Pregnancy, lactation and infectious diseases may precipitate beriberi. McKenzie ascribes one case to hookworm infestation and the loss of blood due to the parasites.

Complications are frequent, particularly infectious diseases. If this happens the cardiovascular disturbance is greatly intensified. Cases of chronic infectious disease are liable to develop beriberi, as has been mentioned, and in these circumstances the nervous disturbances are especially severe and very resistant to treatment. Severe paralyses are common in the beriberi of pregnancy also.

The most serious complication is the development of Shoshin.

Young adults constitute most of the cases. Great weakness, thirst and dyspnea occur with loss of appetite and vomiting. The pulse rate mounts to 120, the respiratory rate 30 p.m. The urine is steadily reduced in amount, precordial pain may appear, the patient is most anxious and restless. Usually these cases show no nervous lesions other than areas of hyperaesthesia in the legs. They run a stormy course and many die within 3 days. Recovery is very rapid under treatment.

Vedder examined patients suspected of latent beriberi by testing for calf tenderness and searching for areas of anaesthesia in the lower legs by means of pin pricks. He then had the suspects squat on their heels. This position is very painful in beriberi and patients frequently are unable to rise without pulling themselves up with their hands.

If a dependable dietary history is available presumptive evidence of beriberi may be secured by calculating the adequacy of the diet by Cowgill's method. The best diagnostic aid at present seems to be the therapeutic test. In acute cases improvement may occur suddenly and dramatically and even the chronic and atypical cases respond promptly to large doses of thiamin.

Certain aids in clinical diagnosis are listed by Shimazono. Nerve tenderness, generalized anaesthesia, severe ataxia, marked difference in involvement in two sides, cranial nerve involvement, disturbed reflexes, foot and patellar clonus and involvement of bladder and rectal sphincters are all absent in beriberi and serve to distinguish it from other diseases.

The injection of adrenalin in cases of mild or chronic beriberi precipitates cardiovascular symptoms similar to those of Shoshin. This has been used as a diagnostic test but great care must be exercised for the response may be of a dangerous degree.

The prognosis in beriberi is now good. Uncomplicated cases seldom die unless of Shoshin attacks which are always a serious matter. In Japan the mortality rate is 2 to 4 per

cent. In Malay Castellani and Chalmers report the mortality to have been 20 per cent. Early diagnosis and prompt medical care are capable of controlling beriberi in all but the most unfavorable cases.

#### SPORADIC FORMS OF THIAMIN DEFICIENCY

It was noted in studying beriberi that other diseases played a conspicuous rôle in modifying the course of the deficiency symptoms. Thus infections and diarrheas precipitate attacks in individuals on the borderline of deficiency and an acute infection occurring during the course of beriberi exaggerates the cardiac dysfunction, while deficiency developing during the course of a chronic, prolonged infectious disease exaggerates the nervous symptoms. Under the latter circumstances the nervous symptoms are not only unusually severe but unusually resistant to treatment. In the discussion of the functions of thiamin reference has been made to the rôle of the other ingredients in the diet on the occurrence of symptoms. It should be quite evident that whether or not a diet is adequate to prevent thiamin deficiency symptoms depends on more than the actual amount of vitamin in that diet. Other dietary factors are important as well as the general health and individual characteristics of the particular person. Because this is true many expressions of thiamin deficiency occur other than the classical beriberi syndrome and since these are important to physicians they will be discussed in detail.

These manifestations are closely related, clinically as well as etiologically, to beriberi, but they present differences which for a long time prevented their recognition as nutritional diseases. What are the predisposing factors which cause them? We can list a number and more may well be found as experience increases.

1. The most important one is the thiamin intake in the sense that if that were ample deficiency would not occur despite the inciting factors. In other words these disturb-

ances appear in individuals whose margin of safety in thiamin allowance has been small.

- 2. The character of the diet is important. First place in this regard must undoubtedly be given to alcohol. The heavy drinker increases his caloric intake so greatly with alcohol that the vitamin calorie ratio is reduced to the danger point. A high carbohydrate intake will do the same thing. Indeed Jolliffe refers to a patient in whom cardiac symptoms seemed to be precipitated by the intravenous administration of 3000 cc. of a 5 per cent dextrose solution.
- 3. Faulty assimilation can produce a deficiency in the face of an adequate intake. Diarrhea is the most common cause of this disturbance. Dann and Cowgill showed, in dog experiments, that diarrhea did not alter the requirements of the vitamin but did affect absorption and that in the presence of diarrhea the oral intake needed to be increased by from 18 to 82 per cent. They recommend that in man the thiamin intake be doubled if diarrhea is present. Malignancy and intestinal fistulae are both capable of altering absorption to the point of causing deficiency.
- 4. Individual variations may be very important. There is considerable evidence that they exist in man and recent experimental evidence that they exist in rats. Light and Cracas have demonstrated this using different strains of rats.
- 5. Advent of an infectious disease. This has been referred to before but Schretzenmayr's views, based on a large experience in Canton, are deserving of comment. Schretzenmayr considers that most of the poor people and soldiers of China are on a borderline of deficiency. However, relatively few develop beriberi, he says, without some precipitating cause, sometimes added deprivation but in most cases an infectious disease, typhoid fever, malaria or pneumonia. Of his cases of typhoid fever 70 per cent developed beriberi and the others had usually been given dietary supplements. Of the cases of intestinal parasitism which he followed nearly two-thirds developed symptoms of beriberi.

Meyer's views on this matter are especially interesting. He believes that thiamin deficiency appears under two distinct clinical syndromes: as a simple polyneuritis and as beriberi (polyneuritis, edema and cardiovascular dysfunction). In most cases of simple polyneuritis complicating factors, usually infectious, precede the nervous lesions. Polyneuritis, he writes, is not early beriberi but a special form of thiamin deficiency. In about half the cases of polyneuritis without edema, heart function is quite normal. In the cases with edema 92 per cent of the cases have heart symptoms. The adrenalin test is negative in polyneuritis but present in typical fashion in beriberi.

It is more generally held at present that these differences are due to the degree of deficiency and the rapidity by which deficiency develops. Thus cardio-vascular manifestations have been ascribed to severe deficiency developing acutely or subsequent to prolonged, partial deficiency (Goodhart).

6. Polyuria and lactation, by exaggerating the normal excretion rate of thiamin, may produce a deficiency.

7. Finally a large number of factors have been recognized which are believed to operate through increasing the metabolic rate: Activity (already mentioned in the discussion of beriberi), delirium, hyperthyroidism, periods of rapid growth and pregnancy. Possibly febrile diseases operate through their effect on the metabolic rate.

Doubtless other factors will be found. A recent report, by Perla and Sandberg, introduces new evidence. They found that manganese in excess increased the thiamin requirement of pregnant rats. Manganese, they suggest, may operate as a catalyst in those oxidative processes in which thiamin is also involved. This may be considered as an example of the type of complication we may expect in the field of "conditioned" thiamin deficiencies.

But whatever the nature of the precipitating factors, the expression of the deficiency conforms in general to beriberi. In other words, neuropathy, cardiac disturbances and edema

result. Indeed Jolliffe states that he has observed all of the reported symptoms of beriberi among his cases. Judging by most reports edema is less frequent than in the oriental disease although Field reports cases of "nutritional edema" in which no other symptoms of deficiency were found and in which the protein intake was adequate. The cases responded to thiamin medication.

# Polyneuritis

Of much greater frequency and, therefore, importance are various forms of polyneuritis. Most of these are cases of alcoholic polyneuritis, the lesions of which have been recognized as identical to those of beriberi for many years (Kimura). The persistence of the belief that alcohol is etiologically responsible is difficult to maintain. Twenty-five years ago it was shown that alcohol does not hasten the onset of beriberi or aggravate its course (Cooper, Vedder). Indeed Meyer has recently observed that alcoholic feedings seemed to delay the onset of symptoms in rat polyneuritis. The belief has, therefore, grown of late years that the polyneuritis found in alcoholics is due to dietary neglect, notoriously true of the type of patient presenting signs of nervous involvement. In addition to this suggestive history and the apparent identity of the lesions, which are of course non specific, the evidence consists chiefly of the beneficial results of treatment, and the presence of frequent transitional cases in which more definite symptoms of beriberi develop (Meyer). Minot, Strauss, and Cobb and many others have reported successful dietary treatment of alcoholic polyneuritis.

Jolliffe and Joffe used Cowgill's formula for establishing the vitamin B requirements of man in cases of alcoholic addiction. Sixteen patients who had, by this standard, been receiving less than adequate vitamin B for twenty-two days or longer showed symptoms of polyneuritis. Four cases having had adequate amounts of vitamin were free of neuritis. They BERIBERI 189

point out that the caloric value of the alcohol must be included in the calculation of the Vitamin/Calorie ratio, for it is often the monopolistic effect of the alcohol which makes the vitamin intake relatively poor.

Wechsler, who has long advocated interpreting alcoholic polyneuritis as due to vitamin deficiency, emphasized the possible rôle of the alcohol as a gastric and hepatic irritant and the likelihood that vitamin deficiency sometimes follows lesions of these organs. Wechsler also considers that the polyneuritis which occasionally complicates pregnancy is due to the pernicious vomiting and consequent vitamin B depletion. Strauss and McDonald have expressed similar views. Their reasons are as follows:

First, the polyneuritis of pregnancy is usually associated with pernicious vomiting; secondly, pregnancy is commonly associated with low gastric acidity; third, experimental evidence also supports the belief that a fetus makes excessive demands on the mother for vitamin B; fourth, clinically and anatomically it is like beriberi and alcoholic polyneuritis and fifth, it is commonest where beriberi is common.

Shattuck writes that Korsakoff, himself, considered that alcohol played but a secondary rôle in the nervous manifestations associated with his name.

The frequency of neuropathy due to thiamin deficiency is considerable among alcoholics. Jolliffe estimates that one-third or one-fourth of 1000 cases of alcoholism had definite symptoms. Of these nearly one-third showed cardiac disturbances as well. The condition has been energetically studied by Jolliffe and his associates. Many cases have been maintained on a basal diet with a vitamin/calorie ratio of 1.7 until their condition was thoroughly assessed. Of 131 cases of alcoholism studied by Romano three-fourths had neuritis and an equal number gave a history of dietary inadequacy.

The usual symptoms are first, heaviness and cramps in the legs, then paraesthesia in feet and fingers and pain in the legs.

Calf muscle tenderness and plantar hyperaesthesia are the earliest objective signs. The latter may extend upwards with sock distribution. Jolliffe classifies these as suggestive signs. A diagnosis is made, when, in addition, the ankle jerks are absent. The cases develop according to the description already given of the clinical course of beriberi. The most severe cases show involvement of the upper extremities, spinal cord or cranial nerves or a "central neuritis."

These signs, in the presence of a predisposing factor and history of dietary deficiency, constitute a rather definite diagnosis. The response to treatment establishes a final one. Strauss and McDonald point out that the clinical diagnosis of nutritional neuropathy from that due to heavy metals is not difficult. The latter affects the anterior horn cells primarily, sensation is little affected, pain is rare and the upper extremities are generally most affected. The nutritional neuropathy commences in the legs with weakness, calf tenderness, burning sensations in the soles of the feet and numbness of the dorsal surfaces and lower ankle followed by climbing hyperaesthesia.

It is well known that gastric disturbances commonly occur in patients with "alcoholic neuritis." Wechsler states that almost all of his cases showed absent or reduced acid. Of 50 cases Villaret et al. found 33 with achlorhydria and 12 with marked hypochlorhydria. There is disagreement as to whether treatment reverses these changes. Voit and Arnold, using crystalline thiamin chloride, found no response.

The polyneuritis of pregnancy is similar. Treatment is very valuable in these cases. Hildebrandt and Otto report the case of a woman with severe paralytic symptoms. She was cured and delivered of a healthy child. The authors caution against interruption of pregnancy for paralytic symptoms until thiamin therapy has been tried. In this case gastric anacidity was also restored to normal by treatment.

The usual chronic alcoholic suffers from a multiple deficiency

although polyneuritis is the most developed and advanced. Nevertheless most of these cases should be searched for signs of pellagra, scurvy and vitamin A deficiency. Ariboflavinosis is a commonly associated deficiency. Cirrhosis of the liver should perhaps be tentatively accepted as a further deficiency effect which is notoriously common in alcoholics (see page 246). Wayburn and Guerard have found cirrhosis and peripheral neuritis to be commonly associated. The association of neuritis was much more frequent among their female patients with cirrhosis than among the males. Improvement was most evident in the patients who received B complex as well as thiamin. There is no evidence that thiamin is involved in the production of hepatic cirrhosis but considerable reason to believe that factors in the B complex are.

Cardiac disturbances due to thiamin deficiency have been present in this country for many years and have, indeed, been recognized for a considerable period. Weiss and Wilkins, however, should be credited with the most complete studies and for emphasizing the frequency and distribution of this disorder. We have already mentioned that Jolliffe estimates the frequency of cardiac involvement in cases with nutritional neuropathy as about 33 per cent. Among 900 case records of patients with some evidence of nutritional disturbance Weiss and Wilkins found 85 they believed suffered from cardiac dysfunction. Within the following two years they observed 35 patients among 5,500 medical admissions. It would seem that the condition is not uncommon and the authors emphasize this by pointing out that in their experience it is a commoner cause of heart disease than either congenital heart disease or subacute bacterial endocarditis.

While the cardiac manifestations do not form a constant syndrome certain symptoms are very common. These are:

1. Dyspnea on exertion with palpitation, tachycardia and embryocardia. The tachycardia may change to bradycardia during treatment. (This is true of oriental beriberi.)

2. Gallop rhythm, prominent cardiac pulsations, pistol shot pulse.

3. Heart of normal size or enlarged. Frequently systolic

and diastolic murmurs.

4. The dyspnea may be very severe and can appear unexpectedly with great severity.

5. Signs of pulmonary congestion.

6. Arterial pressure usually normal with a tendency to increased pulse pressure.

7. Veins in neck may be engorged.

- 8. Skin warm, usually of normal color but may be eyanotic.
- 9. Edema may occur, either dependent or diffuse and is at times severe.
- 10. Patients with severe failure are prone to fever which increases the symptoms.

Jolliffe has stated the case somewhat differently. He groups the cases into one of three categories:

- 1. Patients with edema and serous effusions in the absence of congestive heart failure, enlarged heart or recognized factor producing effusions.
- 2. Edema and serous effusions with signs of heart enlargement and congestive heart failure, and
- 3. Sudden circulatory collapse occurring with or without previous signs of circulatory failure.

Both authors agree that the older observations are correct, namely that patients with slight nervous involvement are more subject to cardiac failure because they are more active.

Jolliffe has listed three further characteristics of the cardiac cases:

1. A mild polyneuritis is present.

- 2. Increased or normal blood velocity in the presence of congestive heart failure.
- 3. Rapid response to specific therapy with complete and permanent reversibility of the circulatory manifestations.

It must be evident that in all those aspects which have been

studied the cardiac as well as the nervous manifestations of thiamin deficiency in this country are the same as those occurring in classical beriberi. In other words the only difference in clinical practice seems to be the difference in terminology which hardly seems justified. This, with a history of an unbalanced diet, the results of the therapeutic test, presence of other signs of deficiency, absence of heart disease of another kind and the changes in the EKG and their response to treatment were indeed the reasons that encouraged Weiss and Wilkins to designate their cases as beriberi.

The similarity extends to other features; males are 4 times as commonly affected as females, the response to the adrenalin test and the appearance of the heart, post mortem.

The EKG observations are not constant or specific. They have been reported by various authors as follows:

Aalsmeer and Wenckebach: Tachycardia and shortened conduction time (P-R interval 0.12 sec. or less).

Scott and Herrmann: Negative T1 and T3. Some with right and some with left ventricular preponderance. Low voltages and slight disturbances in ventricular complexes.

Keefer: Negative T waves, right and left preponderance and low voltages. Weiss and Wilkins: Abnormal T waves, prolonged electrical systole (Q-T) and sinus tachycardia.

Feil: Inverted T waves. Prolonged systole.

Dustin, Weyler and Roberts: Increased electrical systole. Rapid rate. Low voltage and flattening of T wave in first three leads.

This summary is from the report of Dustin, Weyler and Roberts who point out that these changes occur in various other conditions. The EKG records of deficient rats were studied by Zoll and Weiss. The changes were similar to those in human cases but the rate was slow.

Weiss and Wilkins have made a further useful contribution to the subject by calling attention to the effects of inanition on the heart. These are bradycardia, decreased arterial pressure, lowered metabolic rate and presumably decreased blood flow. These changes are entirely different from those due to thiamin deficiency. They must be considered in nutritional cases with cardiac dysfunction since both conditions may cause fatal cardiac failure.

Waring described cases of cardiac enlargement with edema

which responded to thiamin therapy.

## LABORATORY AIDS IN THE DIAGNOSIS OF THIAMIN DEFICIENCY

Thiamin has no properties which lend themselves to delicate testing. The thiochrome reaction is subject to various errors although Westenbrink and Goudsmit reported its successful use in testing urine. The method of Prebluda and McCollum, using as reagent a solution of para-aminoacetanilide and nitrous acid, produces a purple-red compound with thiamin but its application to biological materials is impractical. The amounts of thiamin are, of course, extremely small. Using these methods Schneider and Burger estimate the thiamin content of the urine of normal persons to be between 80 and 100  $\mu$  grams daily and of the blood serum 6.4  $\mu$  grams per 100 cc.

A simpler procedure is Schopfer's test, based on the influence of thiamin on the rate of growth of the mold Phycomyces blakesleeanus. This has been used rather frequently in studies of biological specimens. Its limitations are discussed elsewhere in this volume. Rowlands and Wilkinson, using the Phycomyces test determined the blood level of normal individuals to be between 6.5 and  $16.5\,\mu$  grams per cent. Lower values were found in cases of neuritis, scurvy and malnutrition (3 to  $4\,\mu$  grams per 100 cc.). Other studies of the vitamin excretion have been reported by Harris et al. who used the rat bradycardia method. Good correlation between estimated intake and excretion was secured.

A test which has been used considerably in studying fowl polyneuritis and beriberi is the pyruvic acid test. The bisulphite-binding substances of the blood are determined by a micromethod. This is not specifically due to pyruvate

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but under experimental conditions is quite dependable. and Lu were able to measure the progress of human cases by means of this test. The objection to it in clinical work appears principally to be its limitation to severe grades of deficiency. Wilkins, Taylor and Weiss tested the blood values of many patients and found it nonspecific for thiamin deficiency. Nevertheless the test serves as a useful measure of the disease and with certain limitations as a diagnostic procedure. The pyruvate level in the blood is normally 0.5 mgm. per 100 cc. In mild forms of deficiency the increase may not be considerable but will be excessively increased by muscular exertion and will require an abnormally long time to return to the resting level. Heavy work causes an even greater increase and may precipitate symptoms of beriberi. In severe, fulminating cases of the disease the resting level is high and, according to Platt and Lu, furnishes a measure of the severity of the illness. We have used the method of Bueding and Wortis with success (Appendix A).

Goodhart and Sinclair found blood cocarboxylase to be a good index of the degree of saturation of the body with both cocarboxylase and thiamin. Comparison was made with biological assays. Cocarboxylase was determined by the method of Ochoa and Peters. The blood cocarboxylase is present only in the blood cells. Thus in conditions such as polycythemia erroneous results will occur. In a more recent report Goodhart states that the blood levels for "normal"

boys is  $7.0 \gamma \pm 1.53$  s.D.

#### TREATMENT

The important element in treatment is, of course, the administration of thiamin. Doses of 5 to 10 mgm. daily appear reasonable. This is much more than the calculated daily requirement but it is known that in experimental thiamin deficiency, also, 3 to 5 times the maintenance dose is necessary

to cure. Many cases, especially forms of neuropathy secondary to other diseases will only respond favorably if the dose is still greater and since thiamin is not toxic this is well worth trying.

The response is gratifying and in severe cases dramatic. Hawes states that terminal cases, with pulse rates above 120 per minute undergo an almost immediate response and that changes in blood pressure appear within an hour or two. In such cases, too, large doses are properly given, especially since their effect persists for some days. Cardiac symptoms are said to improve more quickly than the nervous ones.

The other factor in the treatment of severe or moderately severe cases which is of critical importance in the management of cases with cardiac signs is complete bed rest since activity may precipitate sudden attacks and death. Rest alone is enough to induce a considerable degree of recovery in many cases.

And finally diet is important. Jolliffe believes that certain constituents of the B complex are valuable adjuncts. He also gives very practical advice regarding the prescription of a diet. The rule is to eliminate all vitamin-free or vitamin-poor foods. In the severely sick he gives milk, cream, ground liver, puréed legumes, thin, whole grain cereal gruels and fruit juices.

None of the orthodox cardiac drugs are effective in beriberi and even the most powerful diuretics are valueless. Bleeding is recommended for critical cases with heart failure.

We have heard of unfavorable effects from the administration of thiamin in patients with obscure nervous disorders. In most cases the evidence is indirect. However, Steinberg reports 3 patients in whom herpes zoster followed medication and he believed other patients have shown smooth muscle spasms because of thiamin. His cases received from 1200 to 2000 units, some intravenously, others orally. In one case treatment was resumed several months later and herpes reappeared.

## THE RÔLE OF THIAMIN IN OTHER CLINICAL CONDITIONS

Other disorders are believed due to thiamin deficiency or to be related to it. Many of these studies were made before the vitamin was isolated and the sources of thiamin used may have contained other factors. Nevertheless these earlier studies are of considerable interest. In those instances in which concentrates, as of rice polishings, were used the vitamin is referred to as vitamin B<sub>1</sub>.

Mackie and Pound have said that 63 per cent of 75 cases of chronic ulcerative colitis studied by them showed evidence of deficiency disease. Only objective criteria of deficiency states were used. Their study suggested that a relative insufficiency of antineuritic vitamin might account for the abnormal intestinal function they observed after barium meals were given. The evidence could be conversely interpreted to mean that the intestinal disturbance produced deficiency disease by interference with normal absorption in the intestine.

By restricting rats to a given calorie intake over a 100 day period but varying the vitamin B intake Kellog and Eddy showed that those on the higher B intake gave evidence by growth and other signs of much better utilization of the ingested food.

Marks made an extended study of 67 cases in which constipation, colitis, asthenia, and malnutrition were the major complaints. Under supplement of cereal embryo most of the patients reported improved appetites, relief of constipation and cure of symptoms such as nausea, gas, headache, and indigestion. Increased vigor and endurance followed his treatment.

Groen has systematically studied the intestinal absorption of glucose in patients with nutritional disturbances. A segment of the small bowel was occluded by means of a small balloon at the tip of a rubber tube. Measured amounts of glucose were introduced and the rate of absorption measured. The technique eliminates the rôle of diarrhea and directly

measures absorption. Reduced absorption was found in various deficiency states, including polyneuritis, but the part played by other deficiencies could not be controlled and Groen suspected other B complex fractions than thiamin were chiefly responsible. StJulian and Heller found digestion as judged by the coefficient amount ingested — amount in feces amount ingested of protein and fat unaltered in thiamin deficiency.

#### Toxemia

Grüenfelder and Rabinovici attempted to interpret a common, seasonal form of toxemia in infants which occurs in Jerusalem as a form of B starvation. It is a rapidly developing disease which reaches its greatest incidence during the first hot days of Spring and is also common in the hot season that comes in the Fall of the year. Many cases die under the usual treatment which consists of gastric lavage, no food for twenty-four hours, and subcutaneous injections of glucose solutions. Remarkably better results were obtained when concentrates of vitamin B were given intravenously. One hundred rat units were given at a time. Most of the cases responded promptly. Those who had anuria developed a polyuria within four hours and the toxic symptoms gradually disappeared.

Hoobler studied a less violent form of toxemia in infants in Detroit. Many of his patients were rigid and this symptom

in most cases rapidly responded to vitamin B<sub>1</sub>.

Cardiac manifestations do not seem to have been prominent in these series of toxic infants. However, Abt has recently suggested that idiopathic cardiac hypertrophy in infants may sometimes represent unrecognized infant beriberi.

# Pyloric Obstruction

We have been particularly interested in the report of Moore and Plymate in which they describe pyloric obstruction in newly born rats whose mothers had been fed a diet low in



PLATE XII. A diseased ganglion in the pyloric muscle of an elderly subject who had pyloric hypertrophy and whose chief complaint had been persistent vomiting and distension. The nerve cells are degenerate and the ganglion is largely replaced by scar. It is surrounded by hemorrhages. The lesion may be due to vitamin B deficiency.



vitamin B<sub>1</sub>. The condition was cured by feeding vitamin B<sub>1</sub>. Muller found a rapidly increasing tendency to congenital pyloric obstruction in subsequent offspring of rats fed a diet low in vitamin B<sub>1</sub> during their youth.

In the past eight years we have frequently seen examples of pyloric thickening and hypertrophy in elderly subjects. The lesion has only been identified at necropsy and was associated with dilatation of the stomach and a history of repeated attacks of vomiting. Other structural causes, as pressure by the mesenteric artery, etc., were not responsible.

Histological examination showed little of apparent significance other than degenerative changes in some of the nerve cells in the plexuses. However, all of these patients came from very poor homes and in most cases had been living alone or with another ancient. It has been our feeling that such individuals, of whom we see many examples in Grasslands Hospital, are dietary problems, since they live on an extremely small allowance and through lack of interest become less and less demanding in their food requirements. In many of these old persons surprising clinical improvement results if vitamin B concentrates are prescribed. The subject has recently been reviewed by an anonymous contributor to the Lancet.

# The Effects of Vitamin B Supplements on Infants and Children

Many clinical studies have appeared during recent years showing improved growth, appetite, weight, etc., in infants and children following increased amounts of vitamin B<sub>1</sub> in their diets. Studies have been reported by Summerfeldt, Dennett, Morgan and Barry. The results have varied quantitatively but so have the basal diets. Our own results have been fairly typical, and, as they have never been reported may be described here.

In 1931, a subdivision of Grasslands Hospital, a tuberculosis preventorium was opened. It was decided at that time to study the effect of cereal embryo as a dietary supplement.

One hundred and three children were studied for a minimal period of nine months. The ages ranged from three to thirteen years. The children came from the homes of the very poor.

All of these young patients were active at school and play in the Preventorium and only nineteen of them had manifest tuberculosis. The children averaged 8 per cent under weight. The criteria used in studying these patients were the skeletal measurements recommended by the American Child Health Association: Standard hematological examinations, estimation of the extent of dental decay by the Association method, capillary resistance tests, detailed studies of appetite, and x-ray studies of intestinal function. The basal diet to which the cereal embryo was added was an excellent one.

The results of the tests were that the experimental group showed distinct advantages in hemoglobin regeneration—the average was 61 per cent at the beginning of the experiment—and more natural gastro-intestinal function as shown by barium meals. The control group had double the number of cases with gastric retention and less rapid intestinal evacuation than the experimental group.

Knott reported thiamin balance studies in children which showed the body stores in even well-fed children are very low and that concentration can be increased by higher intakes. Based on these tests of saturation the requirement was estimated to be 6 to 7 times the preventive requirement as estimated by Cowgill's method. In a later study Schlutz and Knott demonstrated that a 50 per cent increase in thiamin increased the daily food intake among institutional children.

The advantages of a diet enriched with thiamin (usually B complex) foods has been repeatedly reported. Studies have been published by Poole, Hamil, Cooley and Macy; Robb, Vahlteich and Rose; and Colby et al. Elias and Turner, however, in feeding wheat germ and yeast supplements to healthy children from a poor district found no evidence of improved growth. A general conclusion to be drawn from the

studies we have read is that there are distinct health advantages in a generous intake of thiamin. Hence degrees of partial, sub-clinical, thiamin deficiency are very common. The importance of such a state of partial starvation is believed to be considerable. The studies of Sherman and his colleagues on longevity and stature are applicable here. More particularly does Drummond's report enlighten us. mond sought to reveal the consequences of a partial deficiency maintained throughout the life of the animal. The control animals were fed an adequate diet, the deficient ones were restricted in their B vitamin factors. Their diet was adjusted frequently to maintain weight. More than 1000 rats were studied and the experimental group was divided to permit the results to be followed by two independent groups of ob-Little difference in the incidence of disease was servers. found between the two groups but the frequency of certain lesions was greatly increased. Gastric distension was 5 times as frequent, erosions 3 times, chronic gastric ulcers 3 times, ovarian cysts 20 times and adrenal enlargement 4 times. The duration of life was reduced. Fertility was not greatly influenced but the ability to rear young was strikingly reduced in the deficient group (145 young in the control group and 19 in the deficient one).

While the human requirements as established by Cowgill mark a great advance in the development of an orderly understanding of the dietary factor the requirements for optimal nutrition are not yet known. In rats Williams, Waterman and Keresztesy noted improved size of animals fed larger and larger amounts of vitamin B up to a limit of one hundred times the minimal protective dose. There may be a wide spread in man between the requirements established by Cowgill and the requirements for full growth and vigor.

Thirty years ago Baron Takaki said: "... the percentage of kakke (beriberi) cases was a good indication of the general health of the army, because whenever kakke cases diminished,

the health of the army also improved generally, that is to say, cases of other diseases decreased proportionally."

# Encephalopathy of Wernicke

A characteristic cerebral lesion was described by Wernicke in 1881 which he called acute superior hemorrhagic polioencephalitis. The lesion consists essentially of symmetric petechiae in the wall of the third ventricle, the grey matter about the aqueduct and on the floor of the fourth ventricle. Wernicke believed this the expression of an infectious disease, a polioencephalitis, but recent work suggests that it is an uncommon form of thiamin deficiency.

Alexander has duplicated the lesion in pigeons by thiamin deficiency and similar changes were earlier observed in rats by Prickett. The lesions in the pigeons were located about the ventricular system and consisted of focal degeneration and varicosities of the vessels with associated areas of parenchymal necrosis and frequently of petechiae. They were most common in the paramedian and paraventricular nuclei of the thalamus and hypothalamus, the mammillary bodies, the periductal region of the midbrain, nuclei triangularis and Bechterew of the vestibular nerve and the dorsal nuclei of the vagus. These are the locations of the lesions in man in whom the histopathology is likewise indistinguishable. The lesion has an especial interest in that vascular effects are predominant. Hemorrhagic lesions have been frequently although erratically observed in various organs in thiamin deficiency.

The clinical signs of Wernicke's disease are lethargy deepening to coma, at times with periods of excitement and signs of focal lesions, nystagmus, ophthalmoplegia and Argyll-Robertson pupil. Glycosuria, irregularities of respiration and cardiac function may occur. Vomiting is common. Korsakoff's psychosis may be associated but there is no good evidence that it is due to thiamin deficiency. Many cases have occurred among alcoholics.

At present the evidence that this condition is due to thiamin deficiency consists of the experimental production of the disease in pigeons and its prevention by thiamin, and its frequent association with alcoholism and polyneuritis. On the other hand it has not been reported as a complication of oriental beriberi and the efficacy of treatment in man is unknown. Campbell and Biggart, who have recently discussed the pathology and etiology of the disease quote Tanaka as authority for the observation that it has been seen in Japan among breastfed infants. Alexander suggested that it might represent a complete degree of thiamin deficiency whereas polyneuritis was a manifestation of partial deficiency, the same explanation, it may be noted, proposed by Jolliffe, Bowman, Rosenblum and Fein for a peculiar form of psychosis they believed due to nicotinic acid deficiency and yet which occurred without the classical signs of pellagra.

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## CHAPTER IX

# THE NATURE AND FUNCTION OF RIBOFLAVIN (VITAMIN B<sub>2</sub> OR G)

The vitamin known today as riboflavin or as vitamin B<sub>2</sub> in England and vitamin G in America, is a yellow pigment first noted in milk and described by Winter Blyth in 1879. Its properties were further described by Bleyer and Kallman under the name of lactoflavin in 1925 but they suggested no biologic significance and no explanation of its chemical nature.

The viewpoint that McCollum and Kennedy's water-soluble B contained more than one vitamin was first definitely suggested by Mitchell (1919) and Emmett and Luros proved by autoclaving yeast that they could by this heat treatment completely destroy the antineuritic potency of the yeast without destroying its growth promoting action on rats. This and succeeding work soon established the existence of at least two water soluble vitamins in yeast, one more heat stable than the other; both growth promoting but only the heat labile fraction corrective of polyneuritis.

Next came the suggestion from Goldberger and Tanner (1925) that the heat stable factor was curative of the disease pellagra. Goldberger suggested the following names: Vitamin A-N for the heat labile anti-neuritic vitamin. Vitamin P-P

for the heat stable pellagra-preventive vitamin.

In England and on the continent they later adopted the term vitamin B<sub>1</sub> for the anti-neuritic factor and vitamin B<sub>2</sub> for the heat stable factor. In America we call the heat labile factor vitamin B and the heat stable factor vitamin G.

By 1931 a quantitative method for estimating the relative amounts of vitamins B and G had been worked out in Sher-

man's laboratory; B<sub>1</sub> assay by Chase and Sherman and G assay by Bourquin and Sherman.

But by this time evidence had begun to accumulate indicating that the water-soluble B complex might contain more than one heat stable vitamin and that the heat stable factor detected by the Bourquin-Chase method of assay was not corrective of human pellagra or its analogue, blacktongue of dogs. (Gurin and Eddy; Akroyd; Sure, Smith, and Kik; Koehn and Elvehjem).

In 1932 Warburg and Christian described a new oxidation enzyme derived from yeast which was yellow in water solution and had a greenish fluorescence. They called it the "yellow enzyme." In 1933 Booher in Sherman's laboratory and Kuhn, György, and Wagner-Jauregg on the continent demonstrated that a yellow pigment extractable from whey and egg would provide growth promoting supplement to the Bourquin-Sherman vitamin G-free basal diet. This pigment, called ovoflavin when derived from egg and lactoflavin when derived from milk, was shown by Kuhn and coworkers to be related to Warburg's "yellow enzyme." They also were the ones who suggested the group name of "flavins" for these pigments, later shown present in liver, kidney, malt and other foodstuffs.

Through further study in the laboratories of Warburg, Kuhn, Karrer and Euler it was proven that all of these flavins had a common structure, namely a sugar (ribose) attached to a color nucleus of the alloxazine group (the formula for riboflavin is given on page 23).

The vitamin G detected by the Bourquin-Sherman assay therefore turned out to be a structural component of Warburg's yellow enzyme.

While this identification of vitamin G's chemical nature was developing, progress was also made toward defining the type of dermatitis produced in rats by its absence from the diet. The steps in this study have been reviewed by Hogan who also

contributed significantly to their elucidation. Unlike the lesions of human pellagra or blacktongue, riboflavin deficiency in rats is characterized by a bilaterally symmetrical denudation or loss of hair. There is atrophy of the sebaceous glands, thinning of the epithelium and a glassifying (hyalinization) of the rat tail.

We know, today, considerable concerning the effect of riboflavin deficiency on rats; we know definitely of its essentiality for growth and its inability to correct the specific lesions of human pellagra or blacktongue in dogs. We know, as yet, little of the effect of riboflavin deficiency in human subjects.

#### HUMAN NEEDS FOR RIBOFLAVIN

Spies and coworkers showed that riboflavin and thiamin in baddition to nicotinic acid were frequently necessary to correct collateral deficiencies in pellagrins and in their study of such cases Sebrell and Butler have reported a specific human skin lesion which they call "cheilosis" and believe a specific effect of riboflavin deficiency. Using a diet composed of corn meal, cow peas, lard, casein, white flour, white bread, calcium carbonate, tomato juice, cod liver oil, sirup, and iodide of iron, Sebrell and Butler produced in ten out of eighteen women a pallor of the mucosa in the angles of the mouth. These areas became macerated and in a few days transverse superficial cracks or fissures appeared. The lesions remained moist and were covered with a honey colored crust which could be removed without causing bleeding. The condition was similar to a condition previously noted among children and characterized as perleche.

These lesions did not respond to treatment with nicotinic acid but did respond to treatment with riboflavin in 5 mgm. doses.

## RIBOFLAVIN AND EXTRINSIC FACTOR IN ANEMIA

Another possible rôle of riboflavin was suggested by Castle and has been reviewed by Rhoads.

Castle ate beef muscle, allowed it to digest in his own stomach, removed and neutralized it and fed the mixture to pernicious anemia subjects. The patients were cured. He continued the study to prove that beef muscle alone is inactive; that gastric juice alone is also inactive but that when the two are incubated together something is formed which is curative. He named the substance activated by the gastric juice, extrinsic factor.

The presence of this extrinsic factor has been demonstrated in yeast, muscle, liver, eggs, malt extract, barley, wheat germ and rice bran, all of which, of course, are sources of B complex and of riboflavin.

It is not yet, however, established that riboflavin is the part of the B complex that constitutes the extrinsic factor. Ashford and associates were unable to produce the extrinsic factor by incubating pure riboflavin with normal gastric juice or with the press juice of hog's stomach.

According to Castle the extrinsic factor is water-soluble, soluble in 80 per cent alcohol and acetone, heat stable even in alkaline solution. Its identity with riboflavin is questionable but the question is not yet settled.

#### RIBOFLAVIN AND TISSUE RESPIRATION

To Warburg we owe much of our knowledge of the enzymes concerned in oxidation and reduction in tissue cells. As noted in Chapter III, the yellow enzyme is a member of a very important one of these oxidation-reduction systems. They are of different specificity but all the yellow enzymes consist of a protein combined with a prosthetic group. The complex only is active and the specificity depends upon the protein if the combination, i.e., different cells contain different proteins which may combine with the same prosthetic group to form enzymes of quite different specificity. The yellow enzyme has been shown capable of oxidizing a series of metabolic product (malic acid, citric acid, ethyl alcohol, hexose phosphoric acid

by transporting hydrogen to suitable acceptors. It can also transport the molecular oxygen of the air directly to these substances, something that flavin alone can not do.

That riboflavin is an essential component of the prosthetic group in the cell oxidation enzyme indicates its importance to cell respiration though the daily human requirement to supply adequacy is not known. Adams has shown that when rats receive a diet low in riboflavin the oxygen uptake of their skin is lowered.

The wide distribution of "yellow enzyme" in body tissues and the importance of riboflavin as a structural unit in this enzyme would alone support a claim for importance of dietary supply of this factor to maintain normal tissue metabolism.

It is generally held that before riboflavin can be built into the yellow enzyme it must be phosphorylated. Iodoacetic acid retards ester formation. Laszt and Verzar retarded growth and produced hypertrophic adrenals and alterations in the bones, skin, and blood of rats by feeding a complete diet to which had been added 0.02 per cent iodoacetic acid. The addition of riboflavin to this diet did not restore growth but 0.02 per cent phosphorylated riboflavin (cytoflav) accomplished this effect.

## THE HUMAN REQUIREMENT OF RIBOFLAVIN

With the aid of experimental animals such as rat, bird, and dog the effect of riboflavin deficiency is receiving attention in several laboratories today but at present we have little on which to base the human requirement. Day and coworkers reported production of cataract in rats when deprived of riboflavin. They also produced similar effect in mice, chickens and monkeys and cured it by treatment with pure riboflavin.

Sherman and Langford reviewed human requirements for riboflavin. They pointed out that deficiency of the vitamin stunts the growth of young rats and causes lowering of general tone and a condition of premature aging of the skin with loss of hair. They do not attempt to prescribe either minimal or optimal human requirements but do point out that the optimum need appears to be several-fold that necessary to prevent visible symptoms of deficiency.

Emmerie reports that the daily urinary excretion of male subjects is 30 to 50 micrograms of riboflavin per hour; output however increasing with increase in intake. His findings would indicate that a daily intake of 2 to 3 mgm. of riboflavin (666 to 1000 Sherman-Bourquin units) should compensate for excretion and keep reserves normal in a 70 kilo subject though there is apparently some destruction of riboflavin in the body.

At present writing the U. S. Food and Drug Administration requires prescription of at least 1200 micrograms of riboflavin per day to meet adult needs. Using Booher's figures of equivalence, viz., that a Sherman-Bourquin unit is 3 micrograms of riboflavin, this represents 400 Sherman-Bourquin units. Stiebeling and Phippard (Bull. 507, U. S. Dept. Agric.) suggest 600 Sherman-Bourquin units or 1800 micrograms as a desirable human adult allowance.

Fortunately the substance is quite widely distributed in foodstuffs and is quite stable to heat and hence suffers little destruction in cooking operations.

#### CHEMICAL PROPERTIES

Pure riboflavin is a yellow-orange crystalline product melting at 280°C. with decomposition. It is odorless and soluble in water and alcohol but insoluble in acetone, chloroform or ether.

Crystalline riboflavin protected from light is stable at ordinary temperatures but quite unstable in alkaline solutions.

Riboflavin exhibits a characteristic yellow-green fluorescence; maximum at pH 6 to 8 and decreasing in solutions of less than pH 6 or more than pH 8. It shows absorption bands at 220, 267, 366, and 446 m  $\mu$ .

The fact that riboflavin is light sensitive and its presence in

the retinas of the eyes of various species (Von Euler) suggests a rôle in vision.

According to Kuhn, doses one thousand times the normal given to experimental animals produced no toxic effects.

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## CHAPTER X

# RIBOFLAVIN DEFICIENCY

Reference has been made to "rat pellagra" (page 207) most of the lesions of which were for a time ascribed to riboflavin deficiency. These were described by Goldberger and Lillie in 1926 as follows. The first effect of deficiency was cessation of growth which was followed by a tendency of the eyelids to adhere together with, in some instances, an accumulation of dried secretion on the margin of the lids. About the same time alopecia or loss of fur began and in some animals progressed to almost complete denudation of head, neck and trunk. With or without such loss of fur a dermatitis appeared Lat one or more of the following sites: ears, front of neck and supper part of chest, forearms, back of forepaws, shins, and back of hind paws. This dermatitis was sharply symmetrical. The ears seemed definitely reddened and thickened with what appeared to be a vellow incrustation of dried serum. In healing desquamation took place, leaving the skin of the pinna with a polished, glistening, somewhat parchment-like appearance. In the cases in which the backs of the forepaws were affected, the skin was red and rough and, after healing but before the renewal of the normal, fine, silky hair, the skin had a pale pink glistening, new skin appearance. The backs of the hind paws, when affected, presented at first an appearance as of a matting of the silky fur of this part, and then looked dull and thickened. Later this matted layer of fur began to fissure and crack and then gradually desquamate, leaving a denuded pale pink, glistening skin.

The shortest period for producing this dermatitis was ap-

proximately seven weeks. In addition to the skin lesions they found a stomatitis comparable to that found in pellagra.

No distinctive histologic lesions were found. The dermatitis, which we have since had occasion to examine, consists of a nondescript atrophy with a round cell infiltration of the superficial corium somewhat resembling seborrhoic dermatitis. Smith and Sprunt called attention to the changes in the tail. These consist grossly of thick yellow encrustations ending in atrophy of the skin. Histologically the most striking feature is atrophy of the sebaceous glands. The epithelium is thinned and flattened. Through subsequent studies Smith has shown that this lesion responds to riboflavin although other deficiencies likewise produce it. Whether it is strictly specific or not is uncertain.

György has studied this dermatosis extensively. His original work consisted of supplementing a vitamin B<sub>2</sub> deficient diet with thiamin and riboflavin. These studies clearly revealed that the skin lesions were not due in any considerable degree to flavin deficiency but rather to other constituents of the B complex. The most active factor in producing "rat pellagra" was a water-soluble vitamin which György designated B<sub>6</sub>. However other members of the B group also have anti-dermatosis effect and riboflavin deficiency does account for certain disturbances in the skin; seborrhea with prominent dandruff formation and symmetric loss of hair. A scanty seborrhoic dermatitis is often found. These lesions appear between the 2nd and 4th month of a properly supplemented vitamin B<sub>2</sub> deficient diet. The lesions of B<sub>6</sub> deficiency are described in the following chapters.

A characteristic of riboflavin deficiency in the rat is the appearance of a heavy infestation with lice. This is promptly cured by feeding riboflavin. The phenomenon is demonstrable in robust, vigorous animals and has been ascribed to loss of sensory acuity. Pinkerton and Bessey have found an equally striking effect of riboflavin in that deficiency, in the

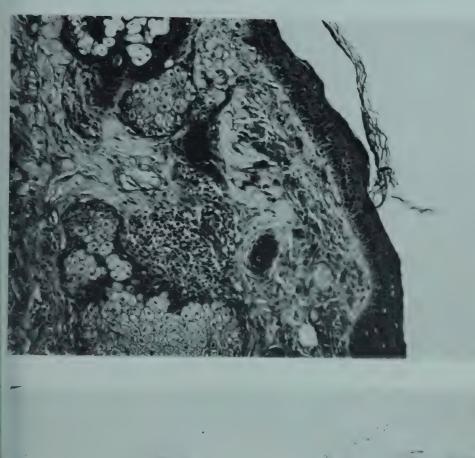




PLATE XIII. Riboflavin deficiency. The upper photograph illustrates the histologic appearance of the lesion in the angle of the mouth in a patient with macerated and eroded angular stomatitis. An area of inflammatory reaction adjoins the sebaceous gland and there are changes in the collagen and elastica causing a granular appearance and clumping.

The lower photograph shows the atrophic skin with scaly stratum corneum and loss of hair seen on the back of rats during riboflavin deficiency.



PLATE XIV. Vascularization of the cornea due to riboflavin deficiency. Photograph of a rat eye which was injected with india ink to demonstrate the plexus of newly formed blood vessels. (Reproduced by permission of Dr. L. V. Johnson and the Archives of Ophthalmology. See Eckardt, R. E., and Johnson, L. V., Arch. Opthal., 21: 315, 1939.)

rat, leads to loss of resistance to the rickettsiae of murine typhus. In the deficient animals the rickettsiae are present in abnormally high concentration in tissues in which they normally are infrequently found or not found at all (see page 453).

The syndrome of sudden collapse, which is a characteristic of prolonged riboflavin deficiency in the dog (vide infra) has not been reported in the rat although we understand it has been observed in an occasional animal.

The most interesting observations of riboflavin deficiency relate to the eye. Bessey and Wolbach describe vascularization of the rat cornea. "It precedes all other demonstrable lesions of the deficiency." Keratitis had been previously observed but attention at that time was directed to changes in the lens of the eye rather than the cornea. Bessey and Wolbach fed their animals a diet composed of 18 per cent casein, 4 per cent Osborn and Mendel salt mixture, 2 per cent cod liver oil, 20 per cent sugar, 48 per cent cornstarch (twice extracted with alcohol) and 8 per cent peanut oil. Thiamin chloride and riboflavin free yeast were given as supplements.

After 3 weeks growth ceased and between the 5th and 7th weeks the palpebral fissure became narrowed, the eyeba!ls sunken, the lids swollen, the tail dry and scabby. The cornea was dull and the skin manifestations of riboflavin deficiency evident. Preceding these signs of deficiency capillaries commenced to grow into the cornea from the vessels of the limbus and within 3 months these newly formed vessels extended more than one-third across the cornea and some reached its center. The vessels were in the form of an anastomosing plexus which lay immediately beneath the epithelium but which later invaded the deeper structures as well. The lesion was rapidly reversed by riboflavin. Turbidity disappeared within 12 hours, within 2 weeks the vascular plexus could no longer be seen clinically although remnants were detected by histological

means for 2 months. Similar changes had been observed in vitamin A deficient rats. The relationship was not clear.

The significance of corneal vascularization and its control by riboflavin were reported independently a month later by Eckardt and Johnson who were studying cataract formation. Only 2 of 12 rats which survived prolonged riboflavin deficiency developed cataract but more than half showed corneal vascularization.

Cataract, reported by Day and associates in almost all riboflavin deficient rats, was rarely encountered in Bessey and Wolbach's experiments. Those instances in which it did occur were believed due to litter susceptibility. Thus in extensive and careful studies two completely divergent results were secured for Day was able not only to produce cataract by riboflavin deficiency but seems to have demonstrated almost as complete control of the process by administering the pure vitamin (Day, Darby and Cosgrove). Other results with similar diets include those of Bourne and Pike who found the incidence of cataract to be 31 per cent, György who found no lens lesions and Richardson and Hogan who "rarely saw cataract." The experience of Eckardt and Johnson has been mentioned. It would seem, therefore, that the consensus is against the rôle of riboflavin in the production of cataract. Galactose rich diets induce cataracts in rats but this does not explain the discrepancy. The galactose cataract is not associated with vascularization of the cornea (Eckardt and Johnson) and is not preventable by riboflavin (Mitchell and Cook). Day suggests that others have not used a completely (flavin) deficient diet.

## CANINE ARIBOFLAVINOSIS

This deficiency disease was first studied by Sebrell and associates. The symptoms, weakness and ataxia, develop rapidly. The animals soon become unable to walk or stand and lie, mentally alert, with spastic extremities. Bradycardia and an

exaggerated sinus arrhythmia are notable features. Respiration is slow and deep. Coma then appears and the animals die within 12 hours, frequently much sooner. Riboflavin produces a rapid cure.

This dramatic collapse developed as a late manifestation of riboflavin deficiency (never in less than 102 days)<sup>1</sup> and was preceded by an inconstant dermatitis which commenced as an erythema followed by a dry and scaly exfoliation. The dermatitis was most common over the chest, abdomen and inner surfaces of thighs and axillae. In male dogs it characteristically involved the scrotum. Anemia was often present but did not respond to riboflavin feeding.

Anatomic study showed fatty degeneration of the liver and, to a lesser degree, the kidney (Henle's loop). Small patches of hepatic necrosis have been found. The bone marrow was atrophic and fatty. Nodular hemorrhagic lesions occurred in the lungs. Extensive but moderately severe degeneration of nerve cells was found in brain and cord as well as myelin degeneration of fibers in the pyramidal tract, median longitudinal bundles and brachium pontis. Myelin degeneration was also seen in the glossopharyngeal and accessorius nerves and in parts of the fasciculus cuneatus.

Similar behavior was noted in B<sub>2</sub> deficiency experiments by Zimmerman, Cowgill and Fox. The etiologic importance of riboflavin in the production of this syndrome was reinvestigated by Street and Cowgill. By using improved diets, deficient only in flavin, they produced the same clinical responses. After 1 to 9 weeks of steady loss of weight and diminished food consumption (which differed from that of thiamin deficiency in its erratic, fluctuating character and the persistence of some eating until the onset of the collapse phenomenon) their animals became apathetic, reluctant to walk, soon staggered and then became semicomatose. Vomit-

Axelrod et al. have succeeded in halving this incubation period by improvements in the basal ration.

ing almost always occurred, some had convulsions and most diarrhea. The diarrhea, however, was a symptom of the collapse stage and was not present during the prodromal period. Complete recovery was effected in 5 of 7 animals within 2 to 3 days by riboflavin treatments. Street and Cowgill demonstrated inversion of the T wave (confirming Sebrell and Onstott), found a fatty liver in one animal but did not observe bradycardia. A pronounced fall in body temperature and respiratory rate occurred during the attacks, the heart rate remaining normal or increasing.

Presumably therefore bradycardia and anemia are due to other than riboflavin deficiency. The syndrome in other respects must have been identical in both laboratories and constitutes a striking clinical manifestation of vitamin deficiency. Whether the dermatitis reported by Sebrell and Onstott must also be accounted for by another deficiency is unknown. As regards the anemia it should be noted that György, Robscheit-Robbins and Whipple found that riboflavin increases hemoglobin production in dogs made anemic by repeated bleedings. The response is of the order of ½ that produced by 300 grams of pig liver.

## AVIAN ARIBOFLAVINOSIS

The importance of the growth factor in the B complex for the chick was demonstrated many years ago and has become recognized as extremely important by the poultry industry. In young chicks the most striking manifestation of chronic riboflavin deficiency is "curled toe paralysis." Prolonged, partial deficiency is necessary for its production. Complete deprivation induces an acute condition marked by paralysis and dystonia which resembles the disease in dogs. The "curled toe paralysis" commences as gradually increasing flexion of the toes continuing to a stage of paralysis. The disorder is usually bilateral.

The lesions are confined to the nervous system (Phillips

and Engel). In 95 per cent of their animals the sciatic nerve was degenerate. Patchy areas of degeneration were also found in the spinal cord involving all portions and tracts. In the legs the nerve end-plates in the muscles are said to be swollen and an occasional muscle fiber is also degenerate but the lesion is otherwise purely nervous.

The eggs of hens partially depleted of riboflavin do not hatch (Engel, Phillips and Halpin) although the animals may be free of lesions. The defect may be restored by injecting riboflavin into the egg on the first day of incubation. The measurements reported by these authors indicate that egg albumin must contain riboflavin in a concentration of from 2 to 3  $\gamma$  per gram to insure normal development of the embryo.

Lepkovsky and Jukes, in extended studies of riboflavin deficiency, lend support to the view previously mentioned that dermatitis is a manifestation of riboflavin deficiency. They failed to produce dermatitis in chicks but did in the rat (in which the lesion was distinguished from "rat acrodynia") and in the turkey. In the latter animal the vent becomes encrusted, inflamed and excoriated. In other respects the disease is similar to that in chicks.

## HUMAN ARIBOFLAVINOSIS

In 1935 Landor and Pallister reported a disease common among prisoners in Singapore and Johore. The signs were an eczematous scrotal dermatitis, glossitis of the tip and margins of the tongue and stomatitis limited to the corners of the mouth. Later on most cases developed paraesthesias, weakness and stiffness of the legs. The mouth lesions developed at the muco-cutaneous junction where the skin became white, sodden and heaped up. Painful fissures appeared. The disease was believed due to vitamin B<sub>2</sub> deficiency because pellagra was not present in typical form and liver, marmite and brewer's yeast were curative.

In 1938 Sebrell and Butler observed a similar lesion in the

mouths of women fed a diet of cornmeal, cowpeas, lard, casein, flour, white bread, calcium carbonate, tomato juice and cod liver oil. Ascorbic acid and thiamin supplements were given. Within 3 to 4 months 10 of 18 women so fed developed pallor of the mucosa in the angle of their mouths. The areas became macerated and in a few days transverse, superficial fissures appeared. The lesions remained moist and were covered by a honey-colored crust which could be removed without causing bleeding. Some of the fissures were  $\frac{1}{2}$  inch long. The condition was obviously identical, clinically, with perleche, a disease previously noted especially among children.

At the same time these patients developed increased redness of the lips (believed due to denudation of the epithelium) and a fine, scaly, slightly greasy desquamation appeared in the nasolabial folds, on the nasal ali and in the vestibule of the nose and on the ears. The lesions did not respond to nicotinic acid but responded promptly to riboflavin.

Subsequently spontaneous cases of the disease were described by Oden, Oden and Sebrell as being quite common in rural Georgia. Three such cases were treated with 5 mgm. synthetic riboflavin. All recovered within a few days. The authors conclude that ariboflavinosis (the name suggested by Sebrell and Butler) is "a common dietary-deficiency disease in the southern states."

Subsequent reports by Spies, Bean and Ashe, Sydenstricker, Geeslin, Templeton and Weaver and Jolliffe, Fein and Rosenblum have confirmed these observations. Smith and Martin, however, treated 4 cases with synthetic vitamin B<sub>6</sub>, given intravenously in 50 mgm. amounts. Their first patient responded within 5 hours. After 24 hours improvement was demonstrable photographically. Treatment was withheld and the lesion recurred after 2 days. Smith and Martin state that experimental B<sub>6</sub> deficiency is more similar to cheilosis than any lesions seen in experimental riboflavin deficiency. They



PLATE XV. Ariboflavinosis. Cheilosis, naso-labial lesion and blepharospasm. (Reproduced through courtesy of Dr. V. P. Sydenstricker and the J. A. M. A.)

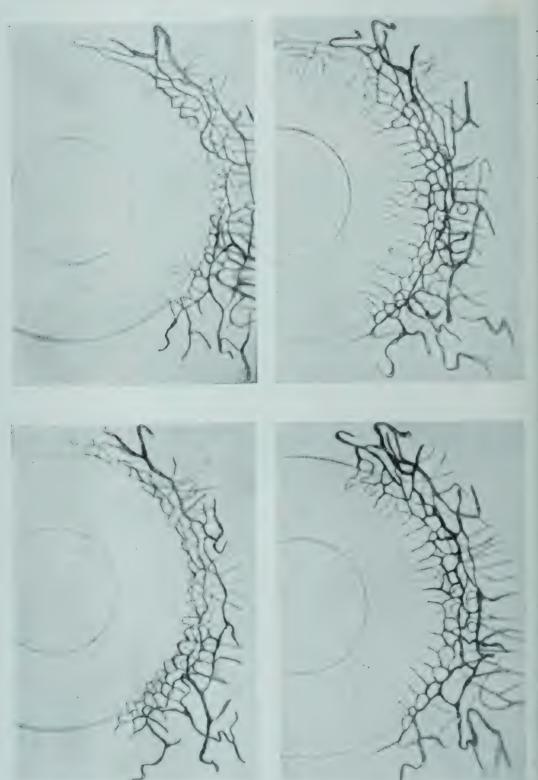


PLATE XVI. Human ariboflavinosis. Drawings based on slit-lamp observations and showing various stages in the develop-

also refer to a report by Aykroyd and Krishnam in which cheilosis was said to respond to treatment with a yeast preparation free of flavin. Smith and Martin suggest that both factors may be necessary for the maintenance of the epithelium of the muco-cutaneous junction or that the two are dependent.

The clinical manifestations of riboflavin deficiency also seem to include other characteristic lesions of the face and tongue. Nasolabial seborrhea, often involving the eyelids and ears is common. A seborrhoic and follicular keratosis of the forehead, malar eminences and chin is described by Sydenstricker, Sebrell, Cleckley and Kruse. Fine filiform comedones over the cheeks and chin resemble "urea frost." These authors confirm an observation of Jolliffe that riboflavin deficiency produces a characteristic glossitis. The features are a clean tongue, of a purple red or magenta color and frequently with fissures. The papillae are large and flattened or mushroom It was formerly thought to be a sign of relapse in shaped. pellagra since it occurs in pellagrins after treatment, either because the deficiency of riboflavin first becomes evident after the cure of pellagra or because the lesion is masked by pellagra glossitis. At times a dry, scaly dermatitis of the hands disappears during treatment with riboflavin. This is an uncommon sign.

Recently it has become evident that the corneal lesions described by Bessey and Wolbach in the rat have their exact counterpart in man. Ocular symptoms and signs had been encountered in various studies of pellagra. They were ascribed to other deficiencies than nicotinic acid; vitamin A and riboflavin. Pock-Steen found the same manifestations in a large group of sprue cases. Of 109 such patients 78 responded to riboflavin. These and other observations of mixed deficiencies, usually associated with pellagra, have been made the subject of special study by Sydenstricker, Sebrell, Cleckley and Kruse. Among 47 cases of ariboflavinosis the symptoms shown in table 24 were encountered. It is evident from this

table that among these particular patients ocular signs and symptoms were more common than the other manifestations

of deficiency.

Circumcorneal congestion had, indeed, been noted by many observers in riboflavin deficiency. Inspection however fails to reveal the characteristic feature of the lesion which is only evident, during life, by slit lamp. By this means the circumcorneal injection may be seen to consist of an extreme con-

TABLE 24
Frequency of Certain Symptoms in Forty-seven Cases of Ariboflavinosis

|                          | Number of patier |
|--------------------------|------------------|
| Photophobia              |                  |
| Burning of eyes          |                  |
| Dimness of vision        |                  |
| Burning lips and tongue  |                  |
| Seborrhea                |                  |
| Cheilosis                |                  |
| Glossitis                |                  |
| Conjunctivitis           |                  |
| Circumcorneal injection  |                  |
| Corneal vascularization  |                  |
| Corneal opacities        |                  |
| Pigmentation of the iris |                  |
| Iritis                   |                  |
| Cataract                 |                  |
|                          |                  |

After Sydenstricker, Sebrell, Cleckley and Kruse, J. A. M. A., 114: 2437, 1940.

gestion of the limbic plexus of vessels with proliferation of new vessels. This stage is followed, if the disease progresses, by radial invasion of the cornea by newly formed vessels just as it occurs in the rat. In many cases superficial nebulae are associated. Interstitial or posterior nebulae are much less common. In advanced cases the penetrating vessels invade the deeper layers of the cornea, but never to the degree that they do in the superficial region.

Prompt response, both of the symptoms and also the signs,

followed doses of from 5 to 15 mgm. of riboflavin. The vascular plexus commenced to shrink within 48 hours. In 1 case the vessels emptied within 24 hours. Extensive lesions required 5 to 18 days to regress.

The order in which these various lesions appear is evident from cases allowed to relapse. The first sign to reappear was conjunctival injection, followed by photophobia and impairment of visual acuity. Corneal opacities were encountered 7 to 10 days after re-instituting a deficient diet. Cheilosis and glossitis seldom recurred before the end of the second week.

In another report on the ocular signs of deficiency Kruse, Sydenstricker, Sebrell and Cleckley state that 2 cases of syphilitic keratitis which were treated with riboflavin also benefitted greatly and the authors raise the question whether syphilis directly causes keratitis or whether it disturbs the riboflavin metabolism.

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# CHAPTER XI

# THE NATURE AND FUNCTION OF OTHER MEMBERS OF THE B-COMPLEX

In 1916 McCollum and Kennedy designated a watery solution containing a growth promoting and antineuritic vitamin as water-soluble B in contrast to fat-soluble A.

As shown in the preceding chapters this water-soluble fraction has already been shown to contain three distinct vitamins whose synthesis and chemical identity have been accomplished, viz.:

(a) Vitamin B<sub>1</sub> or thiamin, the anti beriberi or antineuritic vitamin.

(b) Vitamin B<sub>2</sub> or G or riboflavin, the Warburg yellow enzyme component.

(c) Vitamin P-P or nicotinic acid or amide, the specific anti-human pellagra or anti-blacktongue factor for dogs.

But these three vitamins are not today considered the sole components of water-soluble B. Dr. C. A. Elvehjem has recently tabulated the theoretical and demonstrated members of the B-complex as of present date (table 25). And even this list fails to cover all the water-soluble vitamins whose postulation has resulted from the study of water-soluble vitamin B-complex.

In 1927–28 Williams and Waterman reported the existence of a heat labile vitamin which they called B<sub>3</sub> as essential to weight maintenance in pigeons already adequately supplied with B<sub>1</sub> and B<sub>2</sub>. In 1930 Carter, Kinnersley and Peters reported a B<sub>5</sub> vitamin, heat stable, also necessary for weight maintenance. Carter and O'Brien (1940) have supplied later evidence which appears to indicate that B<sub>3</sub> is identical with

what we now call pantothenic acid and B<sub>5</sub> is identical with pyridoxin. If this is confirmed we need no longer carry these designations (B<sub>3</sub> and B<sub>5</sub>) in our list of vitamin B complex factors.

In 1929-30 Reader described a thermolabile factor in the B complex necessary to prevent a hunched back, lack of coordination and red swollen paws in rats supplied with sufficient B<sub>1</sub> to prevent polyneuritis. She called it vitamin B<sub>4</sub>. Wisconsin

TABLE 25
After Elvehjem from Nutrition, 3: 2, May-June, 1940

| VITAMINS POSTULATED BY NAME  | REQUIRED BY |     |     |         |  |
|------------------------------|-------------|-----|-----|---------|--|
| VITAMINS POSTULATED BY NAME  | Human       | Rat | Dog | Chicken |  |
| Thiamin or B <sub>1</sub>    | +           | +   | +   | +       |  |
| Riboflavin or B <sub>2</sub> | +           | +   | +   | +       |  |
| Nicotinic acid or P-P        | +           | _   | +   | -+      |  |
| Pyridoxine or B <sub>6</sub> | +-          | +   | +   | +       |  |
| Pantothenic acid             | ?           | +   | +   | +       |  |
| Factor W                     | ?           | +   | +   | ?       |  |
| Factor U                     | ?           | ?   | ?   | +       |  |
| Vitamin H or biotin          | ?           | +   | ?   | +       |  |
| Antigray hair factor         | ?           | +   | +   | ?       |  |
| Spectacled eye factor        |             | +   | ?   | ?       |  |
| Adrenal necrosis factor      | ?           | +   | ?   | ?       |  |
| Cartilage factor             | ?           | ?   | ?   | +       |  |
|                              | Ť           |     |     | 1       |  |

N.B.: + means demonstrated to be required; - requirement not yet established.

investigators indicated the factor was required by chicks as well as rats.

Other vitamin B complex factors evolved gradually following a study by Lepkovsky, Jukes, and Krause of what they called the "residuum" or "third factor" necessary in addition to B<sub>1</sub> and G to complete what then was known as the B complex for rat needs. At that time they stated:

It has been found that vitamins B and G (flavin) may be readily removed from a solution such as an aqueous extract of rice bran by means of a rela-

tively small amount of fuller's earth. Further treatments with fuller's earth remove, much less readily, a third factor related to the prevention of rat dermatitis. There remains in solution another factor, the *Filtrate Factor* which prevents chick dermatitis. For convenience, the factor preventing rat dermatitis will be referred to as Factor 1 and the filtrate factor, preventing chick dermatitis, as Factor 2.

This is the first reference and definition in the literature of the term "filtrate factor" in the use of which there is today considerable confusion. As used by Lepkovsky et al. (1936) it apparently refers to the solution filtered off after all B factors adsorbable by fuller's earth have been removed and their Factor 2 is that specifically preventable of chick dermatitis.

Many laboratories today have worked on such filtrate fractions and shown them to contain more than two vitamins when checked against the nutritional needs of various species of animals already adequately supplied with B<sub>1</sub> and G. It would seem, therefore, desirable to discard entirely the term "filtrate factor" and give specific names to vitamins that have been identified as present in the non-adsorbable, residual filtrate after adsorption of the Water-Soluble B Complex. Lepkovsky's Factor 1, that is less readily absorbed than B<sub>1</sub> or B<sub>2</sub>, is known today as B<sub>6</sub> or pyridoxin.

#### VITAMIN B6

In 1934 György applied the term B<sub>6</sub> to that part of the B complex curable of a specific dermatitis developed by young rats fed on a B-complex free diet supplemented by B<sub>1</sub> and riboflavin. Later it became evident that György's B<sub>6</sub>, Lepkovsky's Factor I, Chick and Copping's Factor Y, Hogan and Richardson's and Booher's Factor H referred to the same preventive substance and today it retains György's original designation, B<sub>6</sub> or pyridoxin. Its chemical structure is shown in Chapter II and reveals it to be, like nicotinic acid, a pyridine derivative.

The rat dermatitis for which B<sub>6</sub> is specific is characterized by a symmetrical dermatosis affecting first the paws and tips of ears and nose. These areas become red, and edematous. It is also called acrodynia or florid dermatitis.

As noted in Chapter II, B<sub>6</sub> has now been synthesized in several laboratories and made available in crystalline form. Lep-kovsky claims that dermatitis of the peripheral parts of the body of rats on B<sub>6</sub> deficient diets involving the feet, paws, ears and areas around the mouth, was cured promptly with a daily dose of 10 micrograms; 5 micrograms would clear it up more slowly. This vitamin also produced gain in weight in animals

TABLE 26
Weight Increase in Rats Fed Crystalline B<sub>6</sub> for 14 days

| DAILY INTAKE OF B6 | AVERAGE DAILY GAIN IN WEIGHT |
|--------------------|------------------------------|
|                    | grams                        |
| 25.0               | 3.4                          |
| 20.0               | 3.4                          |
| 10.0               | 3.4                          |
| 5.0                | 2.5                          |
| 2.5                | 2.4                          |

that had ceased to grow on a B<sub>6</sub> free diet. The effect of various dosages is given in table 26.

In contrast to these results Eddy and Dimick found that when rats were placed on a basal diet completely free of B-complex and were given supplements of 85.5 micrograms thiamin, 25 micrograms riboflavin, 50 micrograms nicotinic acid and 20 micrograms crystalline B<sub>6</sub> daily the animals showed no appreciable growth and died in the fourth or fifth week. Vitamin B<sub>6</sub>, like riboflavin, appears to stimulate growth only in the presence of other factors in the B-complex not yet isolated.

Spies, Bean and Ashe, however, have demonstrated a specific

rôle for B<sub>6</sub> in certain conditions exhibited by pellagrins. The following quotation summarizes these observations:

We described recently the study of a large series of undernourished persons who had clinical evidence of pellagra and beriberi and certain symptoms which are corrected by the administration of riboflavin. Such persons are greatly benefitted by the addition of nicotinic acid, thiamin chloride, and riboflavin to their usual inadequate diets. Some of them regain sufficient strength to return to work, thus enabling them to afford a better diet and thereby be restored to good health. Those whose diets remain unchanged develop symptoms which are not corrected by the addition of these synthetic chemical substances. Such symptoms include extreme nervousness, insomnia, irritability, abdominal pain, weakness and difficulty in walking.

Four persons who had been treated successfully for pellagra and beriberi, but who remained on their deficient diets and were now complaining of these symptoms, were selected for treatment. Within four hours after the administration of 50 mgm. pure synthetic vitamin B<sub>6</sub> in sterile physiological solution of sodium chloride, all patients experienced dramatic relief of these symptoms and increased strength. Within twenty-four hours these symptoms had disappeared. One of these persons who had been unable to walk more than a few steps walked 2 miles within 24 hours after the injection of 50 mgm. of vitamin B<sub>6</sub>."

Vitamin B<sub>6</sub> is a derivative of the nitrogen base pyridine, also the fundamental nucleus of nicotinic acid. Birch suggests that it is connected with the utilization of fatty acids.

Birch showed that the unsaturated fatty acids of maize oil were effective in alleviating the symptoms of vitamin  $B_6$  deficiency. He could find no evidence of combination of the vitamin with the lipids but rather suggestion that there was a functional relation between the vitamin and the unsaturated fatty acids.

Fouts proved an intimate relation between B6 and hemoglo-

bin formation, also noted by Chick.

Quackenbusch, Platz and Steenbock reported that rats were protected from acrodynia and continued in good health when maintained on a B<sub>6</sub> deficient diet which was supplemented with

TABLE 27
Rat Units per 100 grams Fresh Substance

| Beef heart | 120<br>130 | Chicken | 0 10 | Wheat germ oil |  |
|------------|------------|---------|------|----------------|--|
|------------|------------|---------|------|----------------|--|

TABLE 28

The Anti-Acrodynic Potency of Foods

(After Schneider, Asham, Platz and Steenbock)

| FOODS                    | UNITS PER<br>100 GRAMS | FOODS                       | UNITS PER<br>100 GRAMS |
|--------------------------|------------------------|-----------------------------|------------------------|
| Lettuce                  | 25                     | Whole wheat bread           | 400                    |
| Spinach                  | 66                     | Oatmeal                     | 330                    |
| Tomato                   | 25                     | Flaxseed                    | 1,000                  |
| Potato                   | 40                     | Rice polish                 | 500                    |
| Carrot                   | 25                     | Wheat germ                  | 1,250                  |
| Beet                     | 13                     | Beef tallow                 | 330                    |
| Banana                   | 66                     | Butter fat                  | 200                    |
| Orange                   | 16                     | Lard                        | 2,500                  |
| Apple                    | 25                     | Linseed oil (comm.)         | 2,500                  |
| Egg yolk                 | 2,500                  | Linseed oil (crude)         | 2,500                  |
| Milk whole               | 40                     | Peanut oil (ether extract). | 5,000                  |
| Milk skim                | 14                     | Peanut oil (benzine ex-     |                        |
| Cheese Cheddar           | 250                    | tract)                      | 5,000                  |
| Beef muscle (raw, dried) | 125                    | Peanut oil (crude)          | 2,500                  |
| Beef muscle (roasted,    |                        | Rice oil (comm.)            | 2,500                  |
| dried)                   | 125                    | Rice oil (ether extract)    | 5,000                  |
| Haddock dried            | 200                    | Soy bean oil (ether ex-     |                        |
| Pork liver dried         | 500                    |                             | 1,000-7,500            |
| Alfalfa leaves           | 600                    | Wheat germ oil (comm.)      | 25,000                 |
| Beans, navy              | 400                    | Wheat germ oil (ether ext.) | 15,000                 |
| Peanuts                  | 1,660                  | Corn oil (comm.)            | 20,000                 |
| Soya beans               | 1,250                  | Dried yeast                 | ,                      |
| Cornmeal                 | 400                    |                             |                        |

N.B.: A unit equals the amount necessary to cure moderately severe acrodynia in 3 weeks.

unsaturated fatty acids either as natural oils or by giving 10 mgm. per day of the ethyl linoleic acid ester.

Dimick and Schreffler found rats deprived of B<sub>6</sub> rarely lived more than 50 to 60 days. A particular effect was complete atrophy of the thymus in the B<sub>6</sub> deficient group of rats and complete absence of fat storage.

György has defined a rat unit of  $B_6$  as the amount necessary to cure the dermatitis developed by rats on a  $B_6$  free diet, the diet adequate in other known factors. On this basis he has reported assays of foodstuffs as shown in table 27.

Wilson and Roy report that cereals are a good source of this factor, while fruits and vegetables, especially orange and tomato, contain little B<sub>6</sub>.

The most complete report on distribution at present writing is that of Schneider, Asham, and Platz. They used the technique of Quackenbusch, Platz, and Steenbock and defined their unit as the amount of source necessary to cure an acrodynia of moderate severity in 3 weeks. Table 28 is taken from their report.

As stated above, B<sub>6</sub> has been chemically identified and was successfully synthesized by Harris and Folker. The compound is stable to strong acids, to alkali, and to nitrous acid. Kuhn found it non-dialyzable from yeast. This fact in combination with its structure may mean that like nicotinic acid and riboflavin it may exist in the cells in combination with a protein and be another part of the cell's oxidation-reduction system.

At present writing we still lack a satisfactory chemical analysis method for quantitative pyridoxin determination.

#### VITAMIN B4

Reader reported a heat labile, water-soluble factor different from B<sub>1</sub>, B<sub>2</sub>, or B<sub>3</sub> which prevented a sort of paralysis in rats characterized by hunched back, lack of coördination and swollen paws. Kline and associates at Wisconsin confirmed the existence of this factor and it has been concentrated but

not isolated from yeast extracts and defatted liver. Human need for the factor has not been demonstrated but it is apparently required by chicks as well as by rats.

# FILTRATE FACTOR OR PANTOTHENIC ACID

As noted in Chapter II, Lepkovsky, Jukes and Krause after separation of B<sub>1</sub> and B<sub>2</sub> from a water extract of wheat germ or rice polish demonstrated in the residuum two vitamins they called Factors I and II. Factor I turned out to be the anti-rat-acrodynia factor B<sub>6</sub>. The factor in the filtrate from the adsorption of Factor I became generally known as Filtrate Factor and was proven corrective of a dermatitis peculiar to chicks.

R. J. Williams in his pursuit of the yeast growth stimulant characterized by Wildier as "Bios" obtained a substance which he has isolated and characterized as pantothenic acid.

Wooley, Waisman, and Elvehjem by a chemical study of the properties of the filtrate factor report that it is probably identical with Williams' Pantothenic Acid and this has been confirmed by Jukes who cured chicks afflicted with filtrate factor deficiency dermatitis with a preparation of pantothenic acid.

Of pantothenic acid, R. J. Williams said to Science Service:

Since its discovery pantothenic acid has been found to be not only present in widely different tissues and organisms but to function as a potent physiological substance stimulating the growth of yeasts, molds, lactic acid bacteria, diphtheria bacillus, protozoa, young alfalfa seedlings and liver worts, and to stimulate the respiration of various tissues. The present discovery of Jukes and of Wooley, Waisman, and Elvehjem is the first one linking it up definitely as a "growth promoting" substance for higher animals, though it has been recognized as a constituent of all types of animal tissue and to be stored in the livers of all animals.

There is evidence that the same substance is required by pigs and dogs and the inference is not a wild one that it is necessary for the nutrition of all the higher forms of animal life and that it makes up an essential part of every living cell. Filtrate factor or pantothenic acid may therefore prove of real importance in human nutrition and the matter appears near of settlement with its chemical identification and synthesis now known. György and Poling have reported experiments that indicate that pathogenic acid may be identical with the anti-gray hair vitamin.

## VITAMIN B7 OR VITAMIN I

Centanni claims to have isolated from alcohol extract of rice polishings a substance which was without effect in the prevention of beriberi or polyneuritis but which prevented digestive disturbances in the birds.

This factor may be identical with that described by Carter and by Rosedale. Little is known of it today and there are no data on its value to humans.

#### VITAMIN H

As noted earlier this letter was used by Booher to describe what is now known as vitamin B<sub>6</sub>. The letter has also been used to describe a substance said by McCay, Bing and Dilley to be necessary to the life of trout. The substance was present in fresh meat, was heat labile and also destroyed by drying the meat. This factor appears to be essential for carnivora such as fox and mink.

Stepp et al. have used the letter to describe a factor heat stable and dialyzable set free from liver by digestion with proteolytic enzymes.

The term vitamin H is today used to describe a factor protective of a type of dermatitis in rats produced by eating uncooked egg white. This factor was first postulated by Parsons and Kelly and the factor was concentrated from liver extract by P. György in 1937. György obtained preparations effective in parenteral doses of 3 to 5 micrograms and suggested that the substance be called vitamin H.

In 1936 Kögl and Tonnis, in their study of yeast growth

stimulation factors, reported the isolation from egg yolk of a crystalline product for which they suggested the name "biotin." In 1933 Allison, Hoover and Burk reported a factor essential for the respiration of certain lower organisms to which they gave the name "coenzyme R." They found this substance essential to the growth of a legume organism known as rhizobia.

György, Melville, Burk and du Vigneaud have reported that these three substances, biotin, coenzyme R, and vitamin H, are closely similar and possibly identical and since biotin has been obtained in crystalline form by Kögl and Tonnis it should be possible to establish whether this similarity is actual identity. This viewpoint is still further emphasized by Porter and Pelczar who were able to show that biotin was a definite factor in the growth production of staphylococcus aureus and they state:

It is of interest that we have been able to replace our biotin concentrate with a preparation of bios II<sub>B</sub>, furnished by Dr. C. N. Frey, of Fleischmann Laboratories, and a sample of vitamin H, from Dr. P. György. This fact lends further support to the recent work of György, Melville, Burk and du Vigneaud, who have indicated that vitamin H, biotin and the coenzyme R factor are probably identical.

The manner in which these particular strains of Staphylococcus aureus respond to the biotin, bios II<sub>B</sub>, or vitamin H concentrates, suggests the possibility of using them for the bio-assay of these substances. Employing such strains in a technique similar to the yeast-growth test of Snell, Eakin and Williams might be advantageous since biotin (bios II<sub>B</sub>, vitamin H) is essential before any detectable growth will occur. Evaluation of such a technique must naturally await experimental data obtained with crystalline biotin.

#### VITAMIN J

Von Euler, Soder and Malmberg reported the extraction of a factor from the juice of fruits that was not antiscorbutic but protected guinea pigs from pneumonia. They called it vitamin J. Its value in treating pneumonia in man has not been demonstrated.

#### FILTRATE FACTORS

The identification of the chick dermatitis factor as pantothenic acid and the availability of this factor in pure form will, it is hoped, permit evaluation of the rat growth factor 2 of Lepkovsky.

#### ANTI-GRAY HAIR FACTOR

In the study of Filtrate factor Morgan, Cook, and Davison noted that rats on filtrate factor deficiency diets showed marked graying of dark hair. Lunde and Kringstad confirmed this observation and claimed that some factor (not B<sub>6</sub>, Filtrate factor, riboflavin, B<sub>1</sub> or nicotinic amide) deficiency caused the black hair of piebald rats to become gray and the white hair of albino rats to become dirty brown. They found the product in yeast and less heat stable than vitamin G.

#### FACTOR W

In addition to B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, and Filtrate factor II, Elvehjem, Koehn and Oleson suggested for a rat-growth promoting factor the letter designation "W". Frost has suggested its possible relation to the pyridine nucleotides.

#### FACTOR U

Stokstad and Manning have suggested the name Factor U for a vitamin apparently essential for chick growth. This factor they found soluble in 50 per cent alcohol, insoluble in ether, acetone, and isopropyl alcohol. It was adsorbable on fuller's heart and charcoal and destroyed in yeast by autoclaving or refluxing for 30 minutes at pH 1.7 to 11. Like W, its significance in human nutrition is unknown.

Elvehjem also describes a rat condition which he calls the "spectacled eye" state. He suggests that it is due to a specific vitamin.

It is a question today whether Factors U, W, and anti-

gray hair factor may not be identical.

Borsook and associates reported a study of the effect of the B complex on 227 cases of functional gastro-intestinal malfunction. The B complex was of distinct value in this treatment and the observers make the following statement:

There are indications from the experimental work on animals and our observations on humans that in most cases the whole B complex is superior therapeutically to any single fraction. There is also the obvious and important economic reason for preferring the whole B complex as it is formed in foods to any highly purified single component.

## OTHER WATER-SOLUBLE VITAMINS POSTULATED

# Factors $L_1$ and $L_2$

Nakahara and associates claim that one may concentrate from beef liver  $(L_1)$  and from baker's yeast  $(L_2)$  substances which are necessary to milk formation. They consider that they function in the maturation of the lactation tissues.

# Factor M

Day, Langston, and Darby report that nicotinic acid is of no value in correcting nutritional cytopenia in the Rhesus monkeys. Combinations of thiamin, riboflavin and nicotinic acid would not correct the lesions. Dried brewer's yeast and liver extract did clear up the symptoms. They have therefore postulated a factor which they designate as "M".

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## CHAPTER XII

# DEFICIENCY DISEASE RELATED TO THE VITAMIN B COMPLEX

"Rat pellagra," as has been pointed out in an earlier chapter, is actually due to multiple deficiency of heat stable vitamin B factors. The dermatitis so designated has not been identical in all laboratories but is principally due to deficiency of vitamin  $B_6$ . The confusion with pellagra stems from the early studies of Goldberger and rested mainly on the fact that pellagra producing diets caused a symmetric dermatitis in rats. Goldberger himself was led to postulate another vitamin, however, to explain the dissimilar nature of the disease in rats and dogs. György's early experiments with supplements of riboflavin and pyridoxin suggested that the number of unknown factors would need to be increased and subsequent work has proven this view to be correct. The morbid manifestations due to fractions of the B complex include, at this time, the following.

# VITAMIN B6 (PYRIDOXIN) DEFICIENCY

The lesions in the rat include a rather characteristic dermatitis of the extremities (acrodynia) and ears. Lesions resembling sebaceous cysts are common about the throat and indolent abscesses about the whiskers. Ulcers occur on the tongue. A seborrhoic condition is present generally, appearing first over the head. There is no pruritus and alopecia is absent or slight. The signs appear after at least one month on the deficient diet.

The histologic changes have been studied by Antopol and

Unna. During the early stages the skin of the paws is denuded, edematous and moist, the ears thickened and scaly, the snout swollen and ulcers common under the tongue. The ears are the tissue of choice in following the sequences of evolution and repair. The lesion commences with swelling of the epithelial cells, widening of the stratum granulosum which is formed of 4 to 5 layers of cells instead of the normal 1 or 2. Hyperkeratosis is pronounced, the stratum lucidum thickened. Intercellular edema and acanthosis occur. On the tips of the ears necrosis is sometimes seen, edema of the corium and hypervascularization of the supporting structures. The cartilage of the ear suffers, the perichondrium loosens and the cartilage becomes disorganized. The edema disappears 24 to 48 hours after a single large dose of pyridoxin. After 3 to 7 days the hyperkeratotic plaques loosen and peel off and the deeper tissues return to normal. Atrophy of the sebaceous glands also responds to vitamin B<sub>6</sub> therapy.

The curative dose of pyridoxin in rat acrodynia is said to be  $5~\mu$  grams (Dimick and Schreffler). Smaller amounts are not uniformly successful. These authors believe that the vitamin enables rats to utilize food more efficiently since on the same basal intake the animals given larger amounts of  $B_6$  were

slightly heavier and contained more body fat.

In the dog deficiency is believed to cause a microcytic and hypochromic anemia. Fouts and associates report that crystalline pyridoxin in amounts of  $60~\mu$  grams per kilogram was curative of this anemia. A similar anemia has been observed in chicks (Chick et al.). These workers observed epileptic fits during the period of deficiency. Jukes describes the signs of vitamin  $B_6$  deficiency in the chick as retarded growth, depressed appetite, poor utilization of food followed by spasmodic convulsions and death. Synthetic pyridoxin was preventive.

Epileptiform attacks have been observed in pigs and rats as well as in dogs and chicks. Chick and associates state that

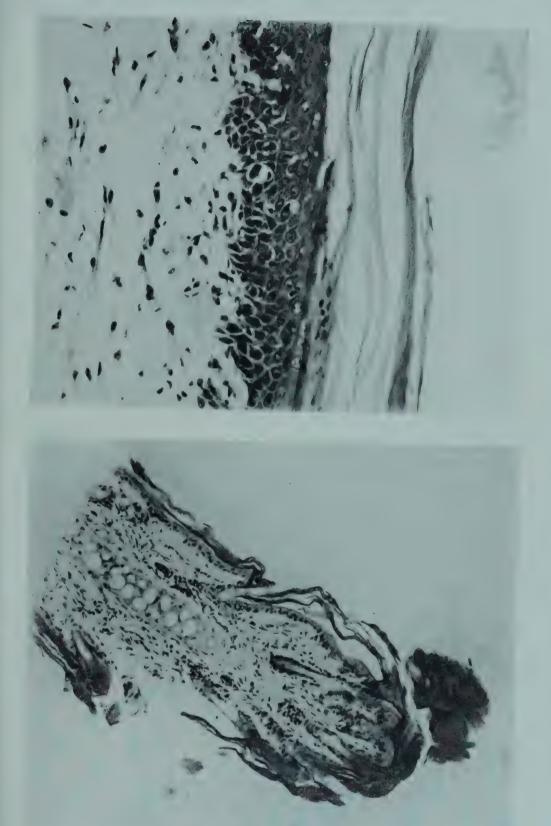


PLATE XVII. Vitamin B<sub>6</sub> deficiency in the rat. Effect of the deficiency on the epithelium. The upper photograph illustrates the acanthosis, parakeratosis and spongiosis which occurs early in deficiency. The lower photograph is from a late lesion which showed thin, pinkish epithelium. The pinna is shown with encrusted adherent scale near the tip. The remaining epithelium is thin and atrophic, the corium congested, the cartilage irregular.



 $P_{\rm LATE}$  XVIII, Vitamin  $B_6$  deficiency. An ulcer of the tongue of a rat fed a deficient diet. This is a common lesion in  $B_6$  deficiency and one that responds to treatment. The inflammatory response is scanty and there is little evidence of repair although the lesion is of several weeks duration.

they appear suddenly. The animals run about in great excitement. They then abruptly fall and pass through stages of tonic and clonic convulsions followed by coma and collapse. Recovery is slow, the animals remaining confused for some time. The fits may last a few minutes or a quarter of an hour. These observations have been confirmed by Wintrobe. Essentially the same behavior was observed in rats.

As a result the vitamin has been used in the treatment of epilepsy as well as other diseases. Jolliffe has reported dramatic relief of the symptoms of Parkinson's disease in particular among cases which had been helpless for less than one year. The rapidity of the response suggested to Spies that vitamin  $B_6$  may exert a sedative effect although the possibility of vitamin deficiency cannot be excluded at this time. Antopol and Schotland secured favorable responses in cases of pseudohypertrophic muscular dystrophy. Kark et al. used  $B_6$  in cases of anemia without response but Vilter and associates induced a slight reticulocytosis in cases of macrocytic anemia.

Spies, Bean and Ashe announce that extreme nervousness, irritability, abdominal pain, weakness and difficulty in walking were observed in several pellagrins after treatment with synthetic preparations, thiamin, riboflavin and nicotinic acid. Very rapid cure of these residual symptoms followed the administration of  $50 \mu$  grams of synthetic vitamin B<sub>6</sub>. Reports of the use of pyridoxin in various diseases are appearing in the clinical literature but at the time of writing nothing of a definite nature is known of the usefulness of this vitamin in the practice of medicine.

## VITAMIN H

The ambiguity which has been associated with the designation vitamin H has been explained in the preceding chapter. The lesion produced by feeding egg white, "egg white injury," consists of cessation of growth after 4 weeks followed by a

desquamative dermatitis which commences in neck and groin and which, according to György, can extend to produce a generalized exfoliative dermatitis. Vitamin H will cure this condition in rats. Vitamin H is probably biotin.

## VITAMIN B4

The syndrome first associated with B<sub>4</sub> deficiency consisted of dermatitis of the paws, muscular weakness and spastic gait. The animals sat in a hunched posture. It was suggested by Keenan and associates that this was the factor responsible for the "nutritional encephalomalacia" described by Pappenheimer and Goettsch some years ago of which the lesions are principally of the cerebellum. The evidence is not yet sufficient. There appears to be a considerable difference in the lesions observed by Keenan et al. and by Pappenhiemer and Goettsch.

## PANTOTHENIC ACID

The rôle of pantothenic acid in animal nutrition is in process of active investigation at the time of writing. From unpublished observations it would appear that in the rat deficiency causes dermatitis of the snout and later of the genitalia and also that it is the principal factor involved in the greying of hair (vide infra). The lesions in the rat consist of sores about the mouth and a scaly dermatitis first seen in the axilla, groin and over the back between the scapulae. In the chick a dermatitis occurs which responds to pantothenic acid. Phillips and Engel state that in addition spinal cord lesions are common as well as involution of the thymus, dermatitis and fatty liver.

# Hemorrhagic Adrenal Necrosis and Pantothenic Acid

A frequent finding in the experiments of György et al. was congestion with patchy hemorrhages of the adrenal glands. Approximately one third of the rats with panmyelophthysis showed such changes. This had been noted by other experi-

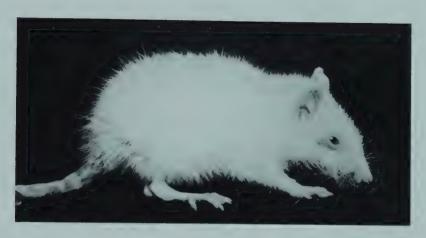


PLATE XIX. Two manifestations of B complex deficiency not due to nicotinic acid, riboflavin, thiamin or pyridoxin. The upper photograph shows the "spectacled eye" lesion formerly considered a sign of "rat pellagra." The lower photograph illustrates the typical position in rat paralysis. (Reproduced by permission of Dr. J. J. Oleson and the Journal of Biological Chemistry. See Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem., 127: 23, 1939.)





meters and was studied by Daft and Sebrell who concluded that it was an independent condition since none of their rats with adrenal hemorrhage had the lesions of panmyelophthysis. The one rat which fulfilled György's description of panmyelophthysis did not have adrenal hemorrhages. Daft and Sebrell observed the lesion in 44 of 72 rats. The inner portions of the cortex were involved, rarely the medulla. Spermatogenesis was greatly reduced. György and associates found the testicular tissue well preserved but no spermatozoa in the lumina of the tubules.

György, Goldblatt, Miller and Fulton called attention to the points of similarity between the disease produced in their rats and the Waterhouse-Friderichsen syndrome (purpura, suprarenal hemorrhage, prostration and a rapidly fatal course).

More recently Daft, Sebrell, Babcock and Jukes report that further supplementing of a B complex deficient diet (with pyridoxine hydrochloride) increases the frequency of adrenal hemorrhage and that synthetic pantothenic acid arrests and causes the lesions to heal. Their experiment included 48 rats on deficient diet. The external signs of disease were nosebleed, ocular exudate, "spectacled eyes" and depilation about the mouth and nose. Among such animals almost all show lesions of the adrenal cortex. If, however, daily doses of  $100 \ \gamma$  of pantothenic acid were fed the external lesions promptly disappeared and when such animals were examined post mortem the adrenal glands were either normal or showed scars, calcification and blood pigment which indicated recovery from cortical hemorrhage and necrosis.

The histological changes in their animals were studied by Ashburn. One of the most striking differences between the adrenals of pantothenic acid deficient and treated animals was their fat content. The depleted animals showed great loss of fat in the zona fasciculata while the treated animals were normal or nearly so. Ashburn points out that since fat is an index of cortical hormone and the animals deficient in

pantothenic acid have other signs of cortical adrenal insufficiency, reduced growth, loss of abdominal fat and retarded testicular function the term "hemorrhagic adrenal necrosis" is not satisfactory and represents only one of the manifestations of pantothenic acid deficiency in the adrenal. Skeletal growth was judged in these animals by histologic examination of the epiphyseal cartilage of the tibia. In depleted animals it becomes extremely thin and epiphyseal bone growth is greatly retarded. Under the conditions of the experiment testicular response to treatment was conspicuous only in the number of spermatozoa. Abnormal spermatids were found in both groups. This may have been due to the relatively brief period of treatment.

#### LIVER CIRRHOSIS AND YEAST

From several separate fields of experimental biology have come suggestions that in yeast and other sources of B complex exists a factor capable of protecting the liver against cirrotogenic substances such as chloroform and heavy metals. Brewer's yeast was reported by Von Glahn and Flinn to be very effective in preventing cirrhosis in rabbits fed lead arsenate. Liver extract was preventive of chloroform or carbon tetrachloride cirrhosis in experiments reported by Forbes. Patek has observed benefits from the clinical use of vitamin B complex in cases of alcoholic cirrhosis. György and Goldblatt describe spontaneous lesions in rats fed the diets used in the experiments previously referred to. These lesions could not be regularly produced at will but occurred in 48 rats supplied with thiamin, riboflavin and pyridoxin. In addition to parenchymatous degeneration, focal and sometimes massive necrosis and hemorrhage certain animals showed perilobular fibrosis. Yeast was preventive.

## NUTRITIONAL ACHROMOTRICHIA

The anti-greying factor was demonstrated in rats by Morgan and Simms. The effect appeared after 8 to 16 weeks on a B



PLATE XX. Nutritional achromotrichia and pantothenic acid. The two rats shown in the photograph were fed for 35 days on a diet deficient in B complex but supplemented with nicotinamide, thiamin, riboflavin and vitamin B<sub>6</sub>. The animal on the left received a daily dose of 100 micrograms of synthetic dextrorotatory calcium pantothenate as well. Both animals were of the same size when the experiment started. Nutritional achromotrichia may be due to others factors as well as pantothenic acid but it is obvious that pantothenic acid has prevented greying in this instance. Photograph through the courtesy of Dr. Klaus Unna.





PLATE XXI. Lesions of the mouth during dietary deficiency. Two extensive lesions observed by Topping and Fraser in monkeys fed diets deficient in vitamin B complex but supplemented with riboflavin and nicotinic acid. (Reproduced by permission of Dr. N. H. Topping. See Topping, N. H., and Fraser, H. F., U. S. Pub. Health Rep., 54: 416, 1939.)

complex deficient diet. The hairs became coarse, lifeless and grey and growth was subnormal. Animals maintained in this state for some months also showed indolent skin ulcers sometimes an inch or more in diameter. Other observations were of a loss of elastic tissue in the corium, atrophy of the hair follicles, atrophy of the testis and a marked atrophy of the adrenal glands. The composite picture was one of senescence.

The first of these manifestations, the greying of hair, has been seen under so many and varied circumstances that it seems improbable that only one factor is responsible. As has been mentioned recent evidence suggests that pantothenic acid is active in preventing and curing grey hair in rats. Oleson, Elvehjem and Hart believe the anti-greying factor is independent of a growth effect.

#### FACTOR M. NUTRITIONAL CYTOPENIA

Day, Langston and Shukers described a nutritional disease in rhesus monkeys encountered while studying B complex deficient diets. The characteristics of the condition are leucopenia, neutropenia and anemia with loss of weight. The clotting time is not prolonged but thrombocytopenia may be a characteristic. Severe ulcerations developed about the gums and mouth and diarrhea was common. The animals died between the 26th and 100th day of deficiency. Riboflavin, nicotinic acid and thiamin did not influence the symptoms. The basal diet was believed to contain adequate amounts of pyridoxin.

The mouth lesions were also observed by Topping and Fraser and studied histologically by Tomlinson. These authors tested various deficient diets for effects on the oral tissues. Vitamin A deficiency produced little effect. Neither did vitamin D deficiency. Scurvy caused severe gingivitis, in several cases generalized, necrotic gingivitis. But of 16 monkeys on a B complex deficiency 12 developed gingivitis, 10 with generalized necrotic gingivitis, 6 with ulcerative lesions and 3 of these progressed to complete, gangrenous

necrosis of the cheek (see Plate XXI). Neither riboflavin nor nicotinic acid controlled these lesions which appear to have been identical to those seen by Langston et al.

#### PANMYELOPHTHYSIS

A striking complication in certain rats on a B complex deficient diet supplemented with thiamin and riboflavin is panmyelophthysis, described by György, Goldblatt, Miller and Fulton. The lesion was associated with the substitution of rice starch in the basal diet by cane sugar but was prevented by using Peter's eluate, a crude B<sub>6</sub> concentrate. More purified preparations of pyridoxin were inactive. Their observations were based on 72 rats which showed the syndrome the signs of which were similar to those of aplastic anemia involving agranulocytopenia, thrombocytopenia and erythrocytemiathus the suggested term panmyelophthysis. The disturbance seemed analogous to the anemia already described in monkeys which was discovered by Day, Langston and Shukers and the anemia in dogs studied by Miller and Rhoads although more This may in part have been due to more effective supplementing of the basal diet.

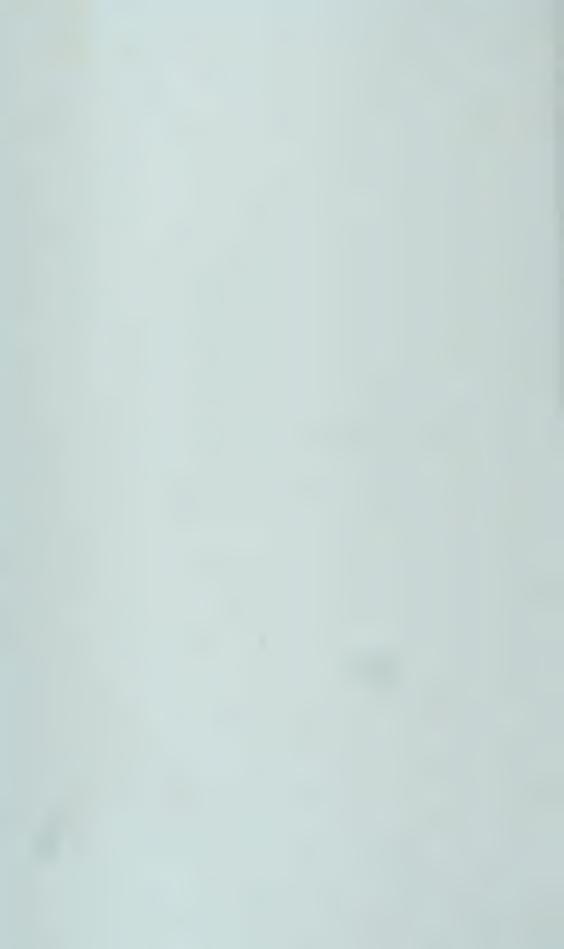
#### MACROCYTIC ANEMIA

It has been widely accepted for some years that the "extrinsic factor" of Castle is commonly associated with the vitamin B complex, as, for example, in yeast. Wintrobe, in reviewing the reports of the use of extrinsic factor finds that roughly one-third of the cases of Addisonian anemia respond to treatment with extrinsic factor. In 15 thoroughly studied cases of Wintrobe brewer's yeast gave maximal response in 5 patients who received 1 to 2 grams per kilogram daily, moderate response in 2 and slight response in 3. The latter cases were but slightly responsive to oral liver. In only 1 patient was liver (orally) more effective than yeast.

A rather close counterpart of Addisonian anemia, but one



PLATE XXII. Purpura, a striking, late manifestation of deficiency in rats with panmyelophthysis. (Reproduced by permission of the Rockefeller Institute for Medical Research and the authors, P. György, H. Goldblatt, F. R. Miller and R. P. Fulton, J. Exper. Med., 66: 579, 1937.)



directly due to lack of extrinsic factor has been extensively studied by Wills. Her attitude at present towards the nutritional agent involved has been stated in a study by Wills, Clutterbuck and Evans. Experimental macrocytic anemia (in the monkey) they conclude, is not due solely to a deficiency of Castle's extrinsic factor for their tests show a lack of parallelism between the effects of various agents in the two conditions. Multiple deficiency may be involved.

Rhoads' studies have shown that Goldberger's pellagra producing diets are capable of inducing in swine a disease remarkably similar to Addisonian anemia, including neurologic disturbances, achlorhydria and stomatitis. It is thus evident that the vitamin B complex is not only of great importance as a source of extrinsic factor but possibly also as a cause of those stigmata associated with lack of intrinsic factor.

Systematization of the deficiency diseases due to deprivation of the vitamin B complex seems dependent on further fractionation and isolation of the substances present. The almost insurmountable difficulties in correlating morbid effects due to various, independently prepared compound fractions is evident in the hundreds of studies of "growth" and "filtrate" factors. The desiderata are (a) the pure substance and (b) the specific morphological or functional effect. When these are available progress is sure and rapid, without them the uncontrollable factors involved in all biological studies, the complications of quantity effects and unrecognized nutrients compounds confusion. It seems best, therefore, to disregard for the time the other manifestations of vitamin B deficiency which are suggested by other studies.

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## CHAPTER XIII

# THE NATURE AND FUNCTIONS OF NICOTINIC ACID (VITAMIN P-P)

Casimir Funk's original rice polishing extract, the original "vitamine" contained some nicotinic acid. Because the latter had no antineuritic effect it was not considered to be a vitamin although in 1937 Funk suggested that nicotinic acid exerted a growth effect when used as a supplement of thiamin. Further pursuit of this viewpoint failed to show a significant response. In the same year, however, Elvehjem, Madden, Strong and Wooley reported that they had obtained from a liver concentrate a substance definitely curative of blacktongue in dogs (a disease apparently identical in origin with human pellagra) and that this principle appeared to be nicotinic acid or nicotinic acid amide.

That this substance was Goldberger's postulated pellagra preventive was promptly confirmed by Fouts et al., Smith et al., Harris, France et al., Spies et al., and Sebrell et al.

#### NICOTINIC ACID AND PELLAGRA

Spies and his coworkers have studied the effect of nicotinic acid, sodium-nicotinate, nicotinic acid amide and coramine upon pellagrins. Treatment produced fading of the mucous membrane lesions, blanching of the erythema of the cutaneous lesions (when present), a tendency toward return of gastro-intestinal function, remission of mental abnormalities (when present), and a diminished excretion of urinary porphyrins.

According to Spies, adequate doses of nicotinic acid, amide or sodium salt will heal the pellagrous glossitis, stomatitis, vaginitis, urethritis and proctitis; early erythematous lesions

are blanched and the porphyrin content of the urine returns to normal. Diethyl amide of nicotinic acid (coramine) has somewhat similar properties; trigonelline, in comparable doses, was inactive. The dosage varied with individuals, 500 mgm. per day usually being effective on oral administration. In a discussion with Ruffin, Spies stated that he considers 500 mgm. in 50 mgm. doses over a period of 24 hours, "A safe, effective and cheap remedy."

The giving of nicotinic acid in oral doses of 200 mgm. or in intravenous doses of 10 mgm. nearly always produces within a minute, dilatation of the small vessels of the skin of the face and upper part of the trunk. This is characterized by flushing, burning, itching and increased temperature. The activity of the sebaceous glands is accelerated. Gastro-intestinal motility may be increased. Changes in respiration, pulse rate, blood pressure and electrocardiogram are regularly encountered. The drug possesses a weak action similar to histamine. It is not destroyed by passage through the capillaries as is acetylcholine.

The early lesions of pellagra resemble sunburn and occur on exposed portions of the body and as noted above, observers have noted that sunlight plays a part in determining the location and occurrence of the lesions. Since, however, lesions can develop in regions fully protected from the sunlight it cannot be other than a secondary cause.

It has also been noted that pellagrous skin lesions occur at the site of the chromatophores where the structure suggests that photodynamic phenomena may occur. It has also been noted that porphyrinemia (increased blood porphyrin) occurs in pellagra and hence that substance has been alleged responsible for the photosensitivity of the skin. Such porphyrinemia (as well as urinary excretion of prophyrins) is also reduced by treatment with nicotinic acid according to Spies.

These facts do not reduce the significance of nicotinic acid as an agent controlling the principal signs of the disease and the use of nicotinic acid compounds is rapidly contributing to the understanding of cases and the differentiation of symptoms observed in individual cases.

We noted earlier that Spies has suggested that pellagra is not simply a dermatitis but "reaction to the lack of important nutritional substances" needed by the tissues of gastrointestinal tract, skin, nervous system and probably of other systems. Does it owe its importance, like riboflavin and thiamin to its place in an essential oxidation-reduction system required by these tissue cells for their normal health and metabolism?

Vilter has utilized the fact that bacilli influenzae require a growth factor "V" which proved to be a codehydrogenase (the cozymase or Coenzyme I of Euler) containing nicotinic acid in the prosthetic group to develop a test for the presence of this V factor or nicotinic acid coenzyme in the blood of normals and pellagrins. The tests showed marked reduction of V factor in pellagrin blood and prompt restoration to normal content by nicotinic acid treatment. However, in their treated cases they noted a decline of appetite and a mild dermatitis developed which was relieved by adding 50 mg. riboflavin for two days. From these results they conclude that the action of nicotinic acid depends upon its synthesis in the body into a nucleotide and ultimately to the cozymase or coenzymes.

Elvehjem reports that in dogs with acute blacktongue the concentration of coenzymes (V factor) was 70 per cent lower in the liver and 35 per cent lower in striated muscle, compared with dogs receiving liberal amounts of nicotinic acid. Kohn and Bernheim report, however, that such determinations in blood of human subjects have little value in the diagnosis of pellagra. Pittman and Fraser could not correlate the amounts of V factor in the urine or blood with the nicotinic acid intake of dogs. Some correlation was found between intake and tissue concentrations. Their

experience emphasized the difficulties in assaying a coenzyme which is so readily inactivated by both heat and certain enzymes.

Such observations would support the view that in nicotinic acid we have a compound essential to the operations of specific body cells as a part of the cell oxidation system, a substance whose absence or inadequacy stops or renders abnormal the essential metabolism of these tissues. The particular lesions and reactions developing in these tissues then become the expression of such abnormal behavior in the tissue cells.

TABLE 29
Nicotinic Acid Content of Foods
(After Elvehjem)

| FOODSTUFF    | ACID PER<br>GRAM DRY<br>MATERIAL | FOODSTUFF          | ACID PER<br>GRAM DRY<br>MATERIAL |  |
|--------------|----------------------------------|--------------------|----------------------------------|--|
|              | mgm.                             |                    | mgm.                             |  |
| Liver, pork  | 1.2                              | Brain, beef        | 0.3-0.5                          |  |
| Liver, lamb  | 1.2                              | Heart, pork        | 0.3                              |  |
| Liver, veal  | 0.9                              | Heart, beef        | 0.3                              |  |
| Kidney, pork | 0.85-1.0                         | Yeast, brewers     | 1.0                              |  |
| Pork loin    |                                  | Yeast, bakers      | 0.5                              |  |
| Pork ham     | 0.4                              | Skim milk powder   | 0.05 - 0.15                      |  |
| Tongue, beef | 0.4-0.5                          | Wheat germ         | 0.05 - 0.10                      |  |
| Veal         | 0.5                              | Dried cereal grass | 0.1 - 0.15                       |  |
|              |                                  |                    |                                  |  |

To date, we have no wholly satisfactory chemical assay method for detection of nicotinic acid but the bio-assay, using blacktongue dogs has already yielded some data. The data in table 29 are reported by Elvehjem using the bio-assay procedure.

Table 30 following is compiled from data of Sebrell representing tests on human pellagrins with various natural foods prior to the discovery of the relationship of nicotinic acid to the disease.

Sebrell found no protection in apples, prunes, salt fat

TABLE 30
Pellagra Preventive Foods
(After Sebrell)

| FOODS              | QUANTITY TO PRE-<br>VENT PELLAGRA | FOODS              | QUANTITY TO PRE-<br>VENT PELLAGRA |
|--------------------|-----------------------------------|--------------------|-----------------------------------|
| Good preventives:  |                                   | Rice polishings    | 400                               |
| Wheat germ         | 150 gm.                           | Peanut meal        | 200                               |
| Buttermilk         | 1200                              | Liver extract      |                                   |
| Beef, canned       |                                   | (Minot)            | Equiv. 100 gm.                    |
| corned             | 200                               |                    | liver                             |
| Beef, fresh lean   | 200                               | Dried brewers      |                                   |
| Chicken, canned    | 325                               | yeast              | 30                                |
| Pork liver         | 24                                | Fair preventives:  | 15 cc. per kilo                   |
| Pork shoulder,     |                                   | Evaporated milk    | 105 gm.                           |
| lean               | 200                               | Dried skim milk    | 30 cc. per kilo                   |
| Rabbit             | 184                               | Fresh skim milk    | 100 gm.                           |
| Salmon, canned     | 168                               | Dried egg yolk     | 340                               |
| Collards, canned   | 482                               | Haddock, canned    |                                   |
| Kale, canned       | 534                               | Beans, kidney red. | 360                               |
| Peas, green canned | 450                               | Beans, Soya        |                                   |
| Tomato juice,      |                                   | Green cabbage,     | 482                               |
| canned             | 1200                              | canned             | 178                               |
| Turnip green,      | 2200                              | Cowpeas            |                                   |
| canned             | 482                               | Mustard greens,    | 533                               |
| Bakers yeast dried | 30                                | canned             | 360                               |
| Bakers yeast dried |                                   | Dried peas         |                                   |
| and autoclaved.    |                                   | Spinach, canned    |                                   |

N.B.: Good preventive means that the quantity given per day cured the disease.

Fair preventive means that quantity used prevented pellagra in most but not in all cases tried.

TABLE 31 (After Elvehjem, 1940)

| ACTIVE   | INACTIVE  |
|--|---|
| Nicotinic acid Nicotinic acid amide Ethyl nicotinate Nicotinic acid N methyl amide Nicotinic acid N diethyl amide Beta-picoline Nicotinuric acid | Pyridine Picolinic acid Isonicotinic acid Nipecotic acid 6-Methyl nicotinic acid Trigonelline 1-Methyl nicotinic acid amide hydrochloride Quinolinic acid Beta-amino pyridine |

pork, cornmeal, cornstarch, rolled oats, rye meal, navy beans, mature onions, potatoes, white or sweet or in gelatin.

Elvehjem reports the forms of pyridine derivatives shown in table 31 as active or inactive. Of chemical tests, Elvehjem also states that the method involving the breakdown of the pyridine nucleus with cyanogen bromide and aniline to give a yellow colored compound which can be measured colorimetrically as most satisfactory of methods to date. The method has been used by Swaminathan, Shaw and McDonald and Pearson.

The substance is stable, non-hygroscopic and its activity is not destroyed by autoclaving. It is soluble in water, 1 part in 100 parts water at 25°C., soluble in alcohol and readily soluble in a solution of alkali carbonates.

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## CHAPTER XIV

#### PELLAGRA

Pellagra may be characterized clinically by dermatitis, stomatitis, gastro-intestinal and nervous symptoms; anatomically by degenerative lesions in these structures; etiologically by a deficiency of a specific dietary substance, the P-P factor of Goldberger or nicotinic acid, nicotinamide or other carboxypyridine derivatives able to replace nicotinic acid in the diet. The disease occurs spontaneously in man. the dog and probably other animals; is widely distributed and in certain districts is a major cause of ill health and death. The name is a corruption of the Italian "pelle agra" or rough skin and was introduced into medical literature by Frapolli in 1771. The earliest comprehensive study of the condition was published in 1735 by Gaspar Casal, physician to Philip V of Spain. A hospital for pellagrins was established in Lugano in 1784. The disease has been recognized in America since 1864 but its prevalence in the South attracted little attention until 1907. The death rate as recently as 1936 was 10.8 per 100,000 persons in the state of Alabama and 200,000 cases have occurred annually in this country.

## THE MODERN HISTORY OF PELLAGRA

The pertinent history of pellagra commences in 1914 when Funk postulated, on theoretical grounds, that pellagra was a deficiency disease. Various facts contributed to his opinion but the conception which seems to have been most important was that the rôle of maize in the diet of pellagrins was probably similar to that of rice in the etiology of beriberi. Funk recognized that maize was not necessarily responsible because

of a hypothetical toxic substance present in it as had been suggested but because it was the cardinal element in a one-sided diet. He further demonstrated that in grinding corn much the same portions of the grain were lost as occurred when rice was milled. Funk's suggestion was seriously tested by Goldberger and other members of the United States Public Health Service. Their feeding experiments in human volunteers, observations in the field and testing of various foodstuffs in Southern institutions thoroughly established Funk's hypothesis. The story of these experiments is fully told in the Bulletins of the old Hygienic Laboratory and need not be repeated here.

The efforts of Goldberger and his associates as well as many other investigators was handicapped by the lack of a suitable experimental animal. Attention was directed to black tongue disease, a common condition among dogs in those localities where pellagra was frequently seen. Dietary and anatomical tests proved this to be the counterpart of human pellagra and black tongue has ever since remained the most satisfactory experimental pellagra.

Experiments were also conducted with rats and led to considerable difficulty since rats also developed lesions resembling those of pellagra. But, the resemblance was not close clinically and the histological lesions of pellagra and black tongue disease were not found in the tissues of rats. The differences between "rat pellagra" and the natural disease became more and more obvious. These studies led to the identification of other vitamins, riboflavin and B<sub>6</sub> in particular, and to the conclusion that the rat does not require the P-P or pellagra preventive factor.¹ The various pieces in the puzzle were rapidly being arranged when the solution came in the isolation from liver of nicotinic acid and the demonstration that it was responsible for the prevention of pellagra.

<sup>&</sup>lt;sup>1</sup> Synthesis of nicotinic acid by the rat has been claimed by Schourie and Swaminathan (Indian J. Med. Res., 27: 679, 1940).

#### THE ETIOLOGY OF PELLAGRA

Pellagra is the consequence of a dietary deficiency of nicotinic acid or related substances. However, various other etiological factors are involved. One which has attracted continuous attention is the influence of sunlight.

The early, acute skin lesions of pellagra resemble sunburn and occur on the exposed portions of the body. This is subject to many exceptions, for example, the vaginal and scrotal lesions. But experiments with protective coverings have generally indicated that sunlight plays a part in determining the location and occurrence of the lesions. Deeks saw an erythematous area on the arm interrupted by the location of an arm band and numerous similar experiences are recorded. Since lesions can develop when completely protected from light the importance of the sun is distinctly secondary. It seems to us that a possible clue to this interesting characteristic of the pellagra dermatitis lies in the location of the skin lesion, occurring, as it does, at the site of the chromatophores where the structure suggests that photodynamic phenomena may occur. Another theory of the effect of sunlight is based on the porphyrinemia which occurs in pellagra and which is blamed for the photosensitivity of the skin.

An etiological factor of uncertain importance at the present time is the multiple deficiency of the pellagra producing diet. Helmer and Fouts have shown by rat experiments that chick antidermatitis factor and riboflavin are both lacking and Margolis, Margolis and Smith demonstrated by dog experiments that both riboflavin and thiamin were present in inadequate amounts. They felt that repeated attacks of black tongue increased the dogs requirement of thiamin.

The same may be true of human pellagra and there is general agreement that the spontaneous cases are quite commonly multiple deficiencies. Lewy and associates, for example, find the nervous lesions are due to thiamin deficiency.

Even the experimental disease in man and many spontaneous cases have shown signs of riboflavin deficiency (cheilosis), and from other parts of the world come reports which implicate vitamin A deficiency as well. The prolonged observation of human cases treated with nicotinic acid reveals the multiple deficiency present in pellagrins. As Kooser and Blankenhorn have said "In this we see the disease pellagra well controlled by nicotinic acid but a considerable amount of poor health persisting."

Other theories of the etiology of the disease are held. Petri, Norgaard and Bandier, in a series of experiments, have seen fit to implicate the stomach. "A disturbance of gastric function, whether of local or central origin has probably a large etiological significance." The bases for this statement are the studies of Petri and his colleagues on gastrectomized dogs and swine and the effect of gastric preparations in the treatment of pellagra. Gastrectomy in mature dogs and swine results in anemia but in younger animals, of both kinds, other signs appear. These are loss of hair, pigmentation, achylia, emaciation, muscular atrophy, splenic atrophy, gelatinous bone marrow, edema, hydrothorax, hyperkeratosis and osteoporosis. The skin lesions, achylia and anemia suggested pellagra. (The other signs are those of inanition). It is interesting to note that Petri found nicotinic acid was incapable of controlling the results of gastric resection. The resemblance to pellagra is not close.

The effect of gastric preparations, as Ventriculin, in the control of pellagra is firmly established and Petri, Norgaard and Bing have observed that cases resistant to the usual dietary treatment may respond to such medication. Mental symptoms did not improve but in some cases gastric acidity returned. Sydenstricker and associates have also reported the extreme effectiveness of gastric preparations in pellagra, and have added other information of value to our understanding of the disease. These workers point out that while

Elvehjem et al. first isolated nicotinic acid from liver it may be erroneous to assume that liver extracts are effective solely because they contain nicotinic acid or nicotin-amides. The methods of preparing liver extracts for use in macrocytic anemia would not husband the nicotinic acid originally present. Yet such extracts are highly effective. Nicotinic acid is not demonstrable in the Cohn fraction, which is, in their experience, especially and rapidly curative of pellagra. Other experiments were made in which extracts from the liver of a pellagrin were found ineffective in curing pellagra although they induced reticulocytosis in Addisonian anemia.

Enough is known concerning the rôle of the stomach in pellagra to justify great caution in dismissing the theories of Petri. That many cases are "conditioned" by lesions in the gastro-intestinal tract or the nervous system is suggested by other evidence (vide infra). These disturbances may be an effect of the deficiency itself and simply constitute a more or less irreversible expression of pellagra. Flinker's studies of the gastric changes in pellagra are pertinent. He has found that a disturbed gastric function regularly occurs in pellagrins and cites his own and others' evidence that this sign precedes all other symptoms. Flinker also found that repeated observations show that the stomach never completely recovers. In other words, gastric function at least would seem to be a characteristic of the predermatitis stage of pellagra and to be irreversible, facts which are in good accord with the lesions Orton and Bender have described.

Sporadic cases of pellagra are frequently "secondary" in nature; presumably the deficiency is not due to faulty ingestion. The cases following colitis, dysentery, gastro-intestinal operations or alcoholism are quite well known and have their counterpart in secondary forms of beriberi. Abbasy states that in Egypt most cases are secondary in that they appear due to poor absorption, the consequence of intestinal parasitism (Schistosomum mansoni and Ancylo-

stoma). In a single family the most heavily infected individuals are the ones which develop pellagra and even the well-to-do who are infected are prone to the disease. Greene has reported two cases of pellagra in patients having myxedema and suggests they were a consequence of the gastro-intestinal dysfunction of the myxedema.

Goldberger studied thoroughly the predisposing causes of pellagra. We may summarize his results as follows: Economic conditions, as they affect the food supply, are of major importance. In the southern states low cotton prices destroy the cotton planters' means of buying food and supply the conditions necessary for the development of the disease. "Under such conditions it has never failed to appear." Factors which interfere with the assimilation of food are of next importance. Among these are loss of teeth, pyorrhea, gastro-intestinal disorders, food fads and voluntary restriction of diet. United States the highest incidence is among adult married women which suggests a relationship between pellagra and pregnancy or lactation. The endemic disease is common among children from 2 to 15 years of age. It may occur in nursing infants. It is no respector of race. Whatever relationships may seem to exist between heredity, hygiene, and pellagra seems due to economic conditions which are associated with poor hygiene and which are commonly perpetuated from one generation to another.

Two other matters concerning the etiology of pellagra may be mentioned here. Jobling and Arnold found a toxic product in cultures of a hyphomycete which was numerically increased in the intestinal contents of the pellagrins. This product produced dermal lesions when its injection was combined with exposure to light. This work seems to us to illustrate possible complexities in the etiology of pellagra which in no way detract from the validity of the deficiency theory since, in common with related observations, they but illustrate pathologic mechanisms possible in persons subject to the

deficiency which may lead to some manifestations of pellagra but not to the disease itself. It has not been claimed that the toxin recovered from their cultures was capable of reproducing pellagra but only one symptom of it, and in the absence of proof to that effect the possible rôle of such toxic material must be considered as of secondary importance. The second matter is the possibility that pellagra is either the direct result of a virus infection, or the result of deficiency secondary to gastro-intestinal tract disturbances due to virus disease of the lateral horns of the cord. The evidence to support these claims is fragmentary, but the theory is an engaging one and serves to emphasize the present divergence of opinion regarding both pellagra and the poorly understood virus diseases. Tucker has advanced the opinion that pellagra is a virus disease because of its epidemic occurrence, seasonal and age group characteristics, and the individual susceptibility noted by clinicians. He also considers the cord lesions to be suggestive of virus infection.

None of these reasons seems very substantial. Nothing comparable to the acute lesions of poliomyelitis or encephalitis has ever been observed in pellagra, and the degenerative changes which do occur are no more characteristic of virus infection than they are of beriberi. The seasonal incidence and susceptibility of certain age groups also occurs in the established deficiency diseases, and in those cases is due to dietary variations or the special requirements of individuals of certain ages. The occasional case of pellagra in which the background does not indicate a restricted diet has often served to arouse doubts of the dietary nature of the disease. Goldberger was aware of this objection. In 1916 he wrote: "We have investigated a number of such cases and have found that though there may indeed have been a rich, varied diet on the family table, the patient, by reason of some personal idiosynerasy, did not actually eat it. In other words, it is assumed in such instances that the diet of the family (or the

institution) is the diet of the individual, an error that is responsible for much of the misconception and confusion in current discussions of the rôle of diet in the causation of pellagra."

The second possibility, that pellagra may at times be secondary to chronic disease of the lateral horns of the spinal cord, is based on the examination of just such an unexpected case of pellagra as we have been discussing. Orton and Bender found older nervous lesions in the lateral horns and suggested that possibly they represented the results of a previous infection, that since they presumably affected the operation of the sympathetic system through the spinal nerves, they might have contributed to the pellagra by causing gastro-intestinal dysfunction.

Smith, Persons and Harvey have lately reopened the possible effect of fuso-spirochetal organisms in producing the mouth lesions of pellagra. The same flora was found in black tongue disease and dogs on the Chittenden, Underhill and Mendel diet (vide infra). This is a perennial theory in oral pathology which does not agree with the facts of the case as we trust the further discussion of the subject will show. The studies of Topping and Fraser (page 247) are in agreement with the almost universally held opinion that these organisms are not important in the production of oral lesions but simply complications of lesions produced by other means.

It will be interesting to note what effect the next ten years' experience with nicotinic acid will have on these various theories of pellagra. The evidence already at hand on the effectiveness of this agent in controlling the cardinal signs of the disease suggests that most, at least, will be relegated to that vast land of forgotten medical theories where good and bad, useful and useless, rest in peace on the shelves of medical libraries.

None of these theories explain the sudden spontaneous re-

missions of symptoms which can occur in pellagra and especially in black tongue. Thus a dog on a basal diet may develop outspoken signs of pellagra and then abruptly recover without change in diet. Under experimental conditions the signs invariably reappear if the deficient diet be maintained.

#### THE MORBID ANATOMY OF HUMAN PELLAGRA

The appearance of persons who have died of pellagra is characteristic only to the degree that appearance of lesions of skin and mouth are characteristic. Emaciation occurs in late cases but the bodies may be in good fat. The viscera, other than the gastro-intestinal tract, give no clue to the nature of the disease present.

Three systems are structurally altered in pellagra: the integument, the gastro-intestinal tract, and the nervous system. Excepting for the early stages of the disease and the skin and colon lesions in this period, the lesions are usually nondescript and throw little light on the pathogenesis. The early skin lesions are therefore of particular interest and value in the anatomical diagnosis of pellagra.

They were originally described by Denton to whom the following account is mainly due. In addition to Denton's own report of his observations, which were made on carefully selected material studied in Panama and in which the autopsies were performed very soon after death, we have reviewed his histologic material and compared it with five cases of our own. Moreover, it has been our practice for some years to examine biopsy material from the margins of pellagrous skin lesions and several cases of this sort have also been reviewed.

## Skin

The first change in the appearance of the skin seems to precede the erythema and, in our experience, has been found immediately adjoining the erythematous patch. The lesion



PLATE XXIII. Pellagra. The early lesions in the skin. Photographs of a biopsy sample showing rarefaction of the corium with fragmentation of some of the superficial collagen.

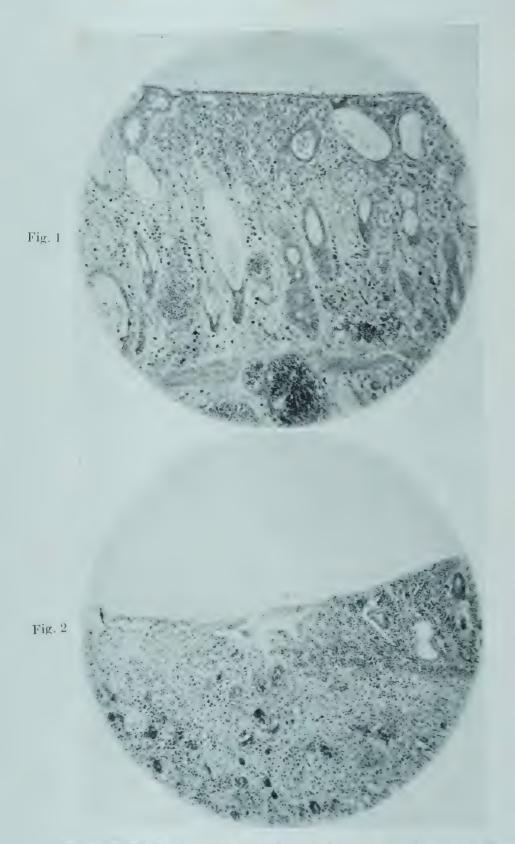


PLATE XXIV. Two lesions in the colon from human cases of pellagra. Figure 1 is a photograph of a specimen from the collection of Dr. James Denton and illustrates the cystic glands which are characteristically found in pellagra, sprue and possibly other related deficiency diseases. The lesion was formerly known as colitis cystica superficialis and was associated with malnutrition. Figure 2 shows the margin of a chronic ulcer of the colon in a case of pellagra of long duration. The ulcers are superficial and shallow.

commences in the corium and consists of edema of the papillae, dilatation of the papillary blood vessels, and deterioration of the superficial—fine collagen—layer of the corium. Slight edema in the deeper portions of the epidermis occurs. Denton saw increased rate of multiplication of the cells of the basal layer as well, but this did not occur in our own cases.

The rarefaction of the corium may be quite pronounced but it is probably evanescent. The sequences have never been accurately worked out because of the paucity of graded material. In well developed lesions the capillary endothelium is swollen and the finer collagen fragmented, often lying in brilliant eosinophilic dots. A few eosinophiles lie in the edematous zone.

It is perhaps significant that the lesion is so sharply limited to the narrow junction zone between the corium and epidermis since this appears to be a region of highly specialized tissues, rich in nerve fibrils and cells related phylogenetically to the nervous tissue—chromatophores. It is possible that damage occurs during the acute stage of the lesion which qualifies the character of the epidermis thereafter.

Rousentoul has described changes in the nerve fibers of the skin in early pellagra. The first change was an intense argentophilia and occasionally spindle shaped swelling of the fibers. The later changes became more pronounced in older lesions in which fragmentation of the axis cylinder was also seen.

Following the early stages of the lesions vesicular formations in the epidermis may occur, and if they do they usually become infected and the epidermis sloughs off, at times in shreds. This in turn is followed by the late stages in which the superficial corium is either atrophic and inconspicuous or may have a thickened horny layer with loosely adherent lamellae. The rete cones are irregular and often elongated and collections of round cells are common in the papillae about the vessels. The character of the pigmentation is disputed. Formerly it was

believed that the chromatophores are increased in numbers and that increased melanin is present in the rete malpighii and basal layer of the epidermis. Herzenberg states that in her experience this has not been so but that many granules of an iron pigment have been present in the epidermis, chiefly in the stratum granulosum.

The oldest lesions are characterized by atrophy of the rete malpighii, the cells of which are also reduced in number and limited in size. The epidermis is thin. The skin appendages

are not affected in pellagra.

The oral surfaces are affected like the skin in the early stages of pellagra and show the same erythema. The buccal mucosa sometimes shreds off in sheets or patches of white necrotic epithelium. The tongue may become atrophic and smooth.

## Gastro-intestinal Tract

A macroscopic lesion of the stomach occurred but once in Denton's cases. In that instance a large false membrane was present near the cardia. In two cases enteritis was found in the ileum and the small bowel was uniformly dark red in color.

The colon, however, was consistently altered, its walls thickened, red in color, covered with patches of pseudo-membrane and stippled with small gray bodies. Histologic examination showed the latter to be cysts formed of distended crypts of Lieberkühn, their lining cells flattened and compressed; their contents retained secretions. The intestinal glands were reduced in number.

According to Herzenberg the cystic lesions are characteristic of pellagra and practically pathognomic. They occur in only one other disease—sprue—and there infrequently. The demonstration of such changes in the colon is therefore of considerable significance to the pathologist since they afford a second basis for an anatomic diagnosis of pellagra. In earlier pathological writings the lesions were identified as colitis cystica superficialis and even then they were recognized as

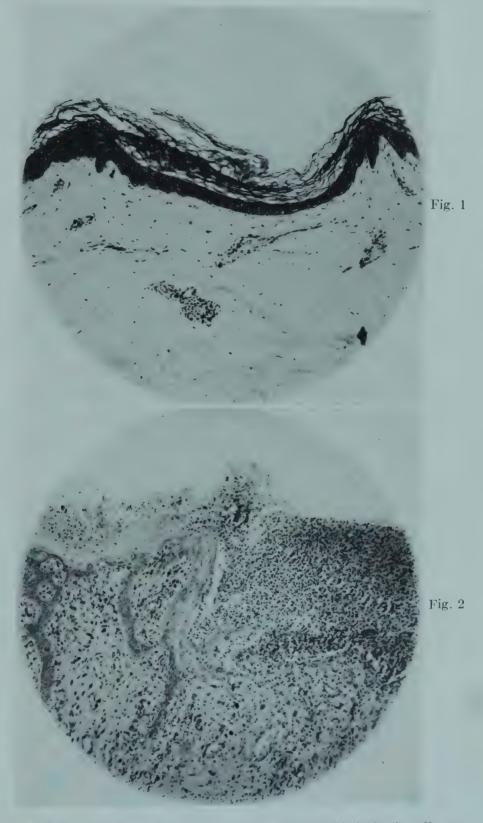
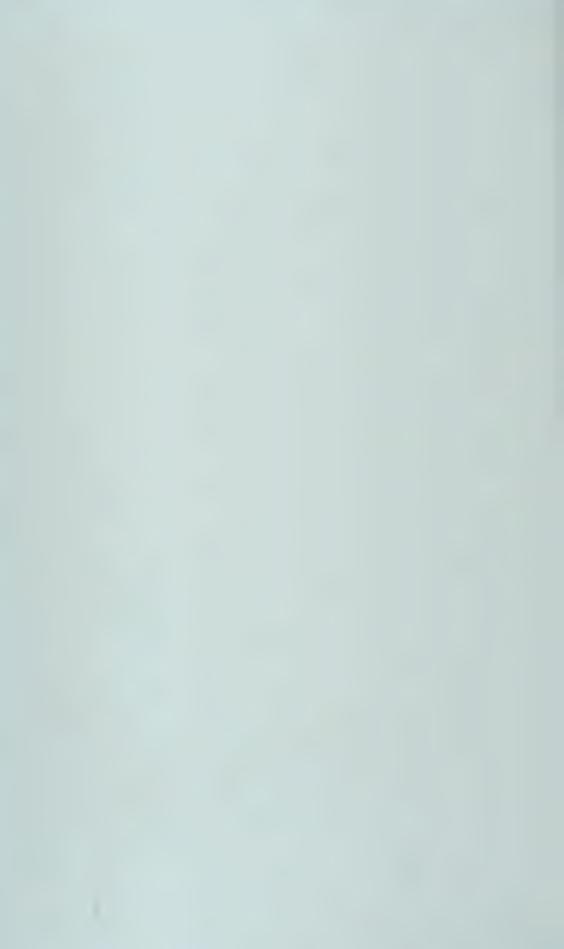


PLATE XXV. Fig. 1. An old, pigmented and atrophic skin lesion in pellagra. Fig. 2. An acute buccal ulcer in pellagra. Membrane lies to the right. The epithelium persists in the left. From a specimen of Dr. James Denton.



being regularly associated with prolonged dietary deficiency. Nothing is known of the mechanism involved in the production of these lesions. They have recently been observed by Lucksch in cases of pellagra, and it is interesting to note that similar changes were described in the fundus of the stomach in Addisonian anemia by Meulengracht.

In Denton's cases the surface of the colon was intact save for patches of superficial necrosis, but the mucosa was infiltrated with plasma cells, lymphocytes, eosinophiles, and endothelial cells. Areas of acute inflammation and hyperemia, and edema were common. Some deterioration of the intestinal ganglia was noted. The late stages of the lesions of the colon are characterized by atrophy of the mucosa and the appearance of superficial, at times cicatrized, ulcers.

## Nervous Lesions

Denton minimized the nervous lesions, considering them a by-product of the disease. This was a natural deduction to draw from his material which was largely composed of acute cases.

The nervous lesions appear relatively late. There is adequate evidence to show that nervous lesions are present in pellagra and indeed may indicate a disturbance predominantly of the nervous tissues of which skin and intestinal lesions are but two expressions. Unfortunately the lesions of the nervous system are not specific, consisting of areas of axonal degeneration of the pyramidal cells of the cortex and degenerative lesions in the spinal cord.

The only difference between the nervous lesions of beriberi and pellagra is the distribution. Central lesions are common in the latter and rare in the former, where peripheral lesions are more pronounced.

The cerebral effects are irregularly distributed, the frontal lobe being said to be more constantly involved than other parts. The large pyramidal cells, in scattered foci, show chromatolysis with displacement of the nucleus and fat accu-

mulations. Hyperplasia of the macroglia may occur.

The spinal cord lesions consist of myelin degeneration of both endogenous and exogenous fibers and are most marked in the posterior columns, especially the columns of Goll. The peripheral portions of these structures are, however, frequently spared. Orton and Bender emphasized the older character of the lesions of the lateral horns in the case they studied. Loss of nerve cells and extensive fibrous glial replacement were present. Tucker also found these areas extensively involved and, since they represent the spinal sympathetic ganglia, the lesions may be related to some of the symptoms of pellagra. Indeed, Orton and Bender suggested that these lesions might be primary and the other manifestations dependent on the dysfunction of the sympathetic nerves, and that this might explain cases of pellagra occurring in well fed individuals.

One characteristic of the lesions of the spinal cord is their irregular distribution. Various tracts may be affected and, since in late cases considerable gliosis may be present, the lesions resemble those of subacute combined sclerosis. It is probable that these two conditions, pellagra and subacute combined sclerosis are related etiologically as well as anatomically. The relationship of the latter to pernicious anemia and its occurrence in cases of pellagra in which achlorhydria occurs, both suggest such a connection.

Lesions in the roots of the cord are infrequent and then usually confined to the posterior roots. In a few cases demyelinating lesions of the peripheral nerves are found.

#### EXPERIMENTAL PELLAGRA

Goldberger, Wheeler, Lillie and Rogers commenced to use the dog for the study of experimental pellagra because a disease of dogs known as black tongue occurs spontaneously

and is similar to human pellagra. Dermatitis has been produced in rats and chicks by elimination of certain vitamin B fractions, but it is improbable that these dermatoses are true analogues of human pellagra.

Shortly after Denton's report on the early lesions in human pellagra, he was intrusted with the examination of the dogs used at the United States Hygienic Laboratories. He examined sixteen animals, at least half of which had early, acute "black tongue," one of which was a normal control and others of which had had recurrent attacks of the disease.

Denton found in these dogs the same distinctive lesions that he had observed in Panama in human cases of pellagra. Lillie has since extended these observations by describing the changes in the viscera not so characteristically affected, and Crane, Lillie and Rhoads, and Zimmerman and Burack have reported anatomical studies of the nervous lesions of "black tongue." Rhoads and Miller have reported special studies of the effect of the black tongue producing diet on the morphology of the blood and bone marrow. The following description is drawn chiefly from these sources.

# The Lesions of Black Tongue Disease

Denton found that the sequences in the epithelium could be most advantageously followed in the mouth. There early lesions may occur before malnutrition is evident in the animal's general condition. On the floor of the mouth or cheeks, or along the inner side of the upper lips, areas of redness appear, at times with slight elevation of the surface. These areas are dark red or greenish gray due to superficial necrosis and membrane formation. When the disease is well advanced the entire lining of the mouth and pharynx becomes deep red, swollen and stippled with patches of false membrane.

The early lesions in the mouth are fleeting; in a few chronic cases they become more persistent. The lesions are common-

est first near the canine teeth, and then may extend as a brilliant red streak completely encircling the alveolar arches.

Once necrosis starts, a fetid odor and drooling appears with stringy, egg white like secretion hanging from the corners of the mouth.

The histologic changes are similar to those seen in human skin lesions, commencing as a thin zone of rarefaction just beneath the epithelium with increase in the thickness of the zone due to the presence of an albumin-poor transudate. The collagen fibrils which form the matrix of this part of the skin become slender and fragmented. The vessels are distended, their endothelium thin. Subsequent changes are secondary to the lesions of the corium and consist of epithelial and cicatrical changes in the corium associated with infiltration by small lymphocytes. Loss of epithelium may result in infection.

Lillie examined the nerves of the lip and found myelin sheath degeneration in from one-sixth to one-third of the fibers in all but two of twenty-six animals.

The acute lesions are difficult to follow on the superior surface of the tongue and in the pharynx, due to structural differences in these tissues. Presumably they are analogous. The esophageal lesions were similar to the buccal ones.

The gastro-intestinal tract shows little evidence of disease on inspection although in some cases it is reddened. More may be learned from histologic examination. The stroma of the villi becomes rarefied and distended with a thin poorly stained fluid. In the colon the lesions are more pronounced, the villi there being greatly distorted and cyst formation in the crypts of Lieberkühn commonly found.

Skin lesions in black tongue seem limited to the skin of the scrotum. Clinically these lesions are similar to the dermal manifestations of pellagra. Denton found microscopic evidence of the disease also.

The nervous system was not comprehensively studied in Denton's work and no significant lesions were found in any PELLAGRA 273

other organs. Lillie found a slight degree of degeneration in the myocardium and in one animal fatty degeneration. The great vessels were normal. Many of his animals had inflammatory lesions in the lungs, a change also present in some of Denton's cases. Lymphoid atrophy is often seen in the spleen. Degenerative changes, of slight degree and extent, may occur in the kidneys.

Dogs which suffer from a chronic, recurrent form of black tongue, develop a striking atrophy of their tongues and sometimes a moderately severe macrocytic anemia which, when it does occur, seems related to the process of black tongue (Rhoads and Miller). The same authors, with a slightly modified diet, produced an acute pellagra like syndrome which at times was associated with more severely ulcerative mouth lesions and extreme leucopenia and a maturation defect of the bone marrow.

The nervous lesions in black tongue are degenerative, as is true of all the lesions due to deficiency of fractions of vitamin B which have been studied. Myelin degeneration and axonal reaction are both found. Both the central and peripheral nerves are affected. The fasciculus gracilis is the most commonly and extensively affected tract (Zimmerman and Burack), but the posterior columns are affected in many cases. These authors considered the lesions to be identical with those in human pellagra. While epithelial lesions may develop in one or two months, the nervous deterioration is relatively late, apparently requiring nearly twice as long.

A frequently recurring observation in the writings of students of experimental pellagra is the variability in the response of different animals. This is much less common in the production of acute black tongue, however, than of the other resulting manifestations of the diets used. Denton's material seems to have run remarkably uniform, especially the group of dogs run on the basal diet alone.

The Chittenden, Underhill, Mendel, "Black Tongue"

A discussion of the pellagra syndrome in dogs would not be complete without reference to a similar stomatitis produced by these workers. The disease was characterized by abrupt onset after a feeding period of from one to eight months. The dogs developed pustules on the inner surface of the cheeks, lips, and on the edges of the tongue after initial symptoms of apathy and refusal to eat. The mouth became so foul and so covered with pustules it resembled rotten meat, and pustules sometimes appeared over thorax and upper abdomen as well.

Anatomical studies made by Lambert showed the buccal mucosa to be red, covered with a fibrino-purulent exudate and the redness to extend over pharynx and upper esophagus. The intestinal tract was injected, particularly the large bowel.

Microscopic study showed the lesions to be very superficial with slight cellular reaction. The first response of the tissues appeared to be hyalin degeneration and swelling of the epithelial connective tissue followed by a sloughing of the epithelium. Lambert felt the intestinal glands might be atrophic.

Underhill and Mendel illustrated their report with pictures of the gross appearance of the mouth lesions which closely resemble those seen in black tongue. However, no photomicrographs are available by which the minute structure of the epithelium may be compared with pellagra and, as Goldberger has pointed out, scrotal lesions did not occur in Underhill and Mendel's animals as it commonly does in black tongue. Moreover, the dermatitis on the chest is foreign to the latter.

Since carotene completely controlled and prevented the Underhill-Mendel lesions, and since proof of the identity of the two conditions is lacking, it seems probable that the two diseases are essentially different though grossly similar. The syndrome would seem to be very similar to the combined deficiency of vitamins A and B which has been described in man by Wright who has designated the syndrome "polyavitaminosis A and B." The histologic criteria should be applied to all future

forms of experimental pellagra for much confusion now exists which microscopic study could presumably clarify.

# Pellagra in Pigs

The Goldberger-Wheeler diet is capable of producing a pellagra-like condition in pigs. Young animals develop loss of appetite, retarded growth and a slowly developing diarrhea. Their skin becomes scurfy, with scabby patches especially common on the backs of the ears. Death usually occurs within one month after the appearance of the diarrhea and post-mortem examination shows a pronounced inflammation of the caecum and other parts of the colon with a plastic exudate. Glossitis and stomatitis do not occur. The condition has been studied by Birch, Chick and Martin. These authors report that, while the colitis is intense enough to suggest infectious disease, yeast is curative.

Pig pellagra has been frequently used in the study of the disease. It assumes added interest in view of the experience of Madison, Miller and Keith who investigated an unthrifty herd of pigs in Pennsylvania. Of 76 animals 40 had died. The survivors were listless, ate poorly, suffered from diarrhea, and had a heavy, scurfy dermatitis of the body and ears. The similarity of the condition to the experimental disease in pigs led to a trial of nicotinic acid in daily doses of 50 mgm. Prompt recovery occurred. Presumably pellagra occurs naturally in the pig as well as the dog.

Harris has fed monkeys a similar diet. Within a few days to a week the animals became patently ill, appetite failed and a profuse diarrhea soon followed. Some dryness and roughening of the skin was noted. 5 to 25 mgm. of nicotinic acid daily was curative. Clark has described a small experiment using monkeys in which the diet used was copied from the dietary associated with pellagra in Egypt. Within 2 months the experimental animal became weak, the palms of the hands were swollen and red, a rash appeared on the abdo-

men and around the eyes (especially on the eyelids). The control animal remained well on the same regimen plus dates. Neither of these studies of pellagra in the monkey can be considered complete or conclusive although Harris' results strongly suggest that the monkey is susceptible to nicotinic acid deficiency.

Contrarily lambs thrive on a black tongue producing diet according to Pearson, Schmidt and Mackay. The same situation seems to exist in the rat although this may be but partly true and the requirements of the pellagra preventive be simply extremely small. The study of rat "pellagra" forms a large part of the work which has been done in investigating vitamin B complex. Goldberger early turned to the rat as an experimental animal. The pellagra producing diets were found to induce a condition resembling pellagra and characterized by a prompt slowing of growth followed by a dermatitis, usually manifest after about two months, beginning on the chest and neck, spreading to the lateral aspects of the forelegs, paws and lower jaws. The skin becomes reddened and the hair falls out. The fairly constant symmetry of the lesions, inflammation of the mouth and occasional diarrhea, all suggested pellagra. Furthermore the foods and yeast extracts which cured "black tongue" and pellagra likewise cured the rat disease and Goldberger was led to consider the condition to be pellagra. It is of considerable interest that Denton was unable to confirm the diagnosis histologically although he immediately recognized the identity of "black tongue" and pellagra. Subsequent study of rat "pellagra" has thoroughly proven that it is not pellagra at all but rather the result of other B factor deficiencies, chiefly vitamin B6, which has been described elsewhere in this volume. Whether the condition produced by Gurin and Eddy, who supplemented a deficient diet with beef extract and a neutral autoclaved yeast must be considered as genuine pellagra is uncertain since the observations have not been repeated. It is PELLAGRA 277

worth noting that lesions having the histologic characteristics of those of pellagra were found in their rats by Denton who was likewise able to separate the experimental animal tissues from those of controls deficient in rat dermatitis factor on histological evidence.

The skin lesions studied by Salmon and Guerrant in rats fed diets low in vitamin B complex would also seem to be unlike pellagra. These lesions commenced as vesicles which evidently itched considerably and were rapidly transformed into superficial pustules from which a gram positive coccus could be cultivated. The organism was not pathogenic for normal rats but reproduced the original lesions early in the course of a vitamin B complex deficiency. The descriptions of the lesions hardly warrant the assumption that they resemble pellagra and the experiment of Salmon and Guerrant is significant only in indicating the lowered resistance to a pyogenic organism of rats fed on vitamin B complex low diets.

### THE DIAGNOSIS AND TREATMENT OF PELLAGRA

# Symptoms

Goldberger considered the older classification of pellagra as acute or chronic to be misleading. The disease runs a variable cycle however, and a distinction between different forms may be justified. It seems unlikely that any fundamental differences exist between the acute and chronic cases and intermediate or transitional forms are frequent. The most acute cases develop rapidly, may have a slight fever, rapidly become soporific, and terminally resemble—except for the absence of pupillary contraction—opium poisoning (Denton). The other extreme is seen in those cases with recurrent attacks every Spring for ten or twelve years with constantly increasing mental and nervous symptoms and finally death.

The earliest manifestations of pellagra constitute a prodromal period which is rarely absent. Goldberger considered the most constant early expression of the disease to be loss of weight and some loss of strength in the lower extremities. Insomnia, lassitude, vertigo, headache, anorexia, and diarrhoea are all common symptoms of the prodrome.

Somewhat later, burning sensations in the mouth appear, at times associated with redness of the tip or tip and margins of the tongue. The stomatitis is very painful and associated with excessive salivation.

Few pellagrins fail to give a history of gingivitis and stomatitis according to Kirkland. Frequently patients first report to a dentist and a very characteristic complaint is that their mouth feels scalded. The gums are tender and eating and drinking painful. Aphthae may be present on the gums or cheeks and are significantly resistant to the usual treatment with caustics. After several weeks of these symptoms the mouth improves but changes may persist in the tongue and Kirkland has found many cases in which he correctly suspected that pellagra was present in remission from the appearance of the tongue.

During active pellagra the tongue is often swollen and tremulous, sometimes cyanotic. Fissures and ulcers may occur on tip and anterior margins. The most characteristic lesion, as has been mentioned, is the shedding of epithelium which begins at tips and sides and may either be confined to these portions or extend widely.

The lesions in the corners of the mouth, inconstantly present, are due to riboflavin deficiency and not to pellagra.

A feeling of heat in the stomach may occur and three-fourths of all cases complain of diarrhoea. Nausea and vomiting with a desire to eat but regurgitation of whatever food is eaten are later digestive symptoms. Many cases (40 per cent) develop achlorhydria.

In persons with such symptoms the presence of the skin lesions is ample evidence of the presence of pellagra. The diagnosis can never be made with certainty in the absence of the dermatitis. The most important features of the skin



PLATE XXVI. Pellagra. Upper photographs show dermatitis of face and hands. The lower photograph shows Casel's necklace.



PLATE XXVII. Pellagra. Dermatitis which developed on the hands of a prisoner.

manifestations are the sharp margins, the bilateral symmetry, the manner of their evolution, and the pigmentations and keratosis.

The lesions commence as dark red areas which become confluent and gradually browner in color. Scaliness develops during the erythematous stage with thickening of the skin and sometimes bullae and vesicles. Burning and itching may be associated with the early lesions. The latter stages show a tendency to atrophy with a senile, parchment texture of the skin.

The distribution of the lesions is characteristic. The commonest sites are the dorsal surfaces of the hands, and lower forearms, and the neck. The former has a gauntlet pattern. On the neck a necklace formation is frequent (Casel's necklace). The dorsum of the feet and lower legs are sometimes affected in persons habitually barefoot. There may be patches over the sternum and on the labia or elsewhere in which cases the lesions are usually associated with mechanical irritation.

The scrotal lesions of pellagra require special mention because they indicate a current source of uncertainty in our knowledge of the disease and also because they have historical interest. Goldberger and Wheeler's experimental human pellagra, produced at the Rankin Farm of the Mississippi State Penitentiary, was noteworthy for the frequency of this lesion. Of the 11 volunteers fed the experimental diet, 6 developed skin lesions. All of these had lesions of the scrotum and Goldberger and Wheeler were led to believe that scrotal lesions were the earliest dermal manifestations of the disease. At that time only 2 reports of such lesions existed, one by Stannus, writing from Nyasaland, Africa. Stannus found, among 100 cases of pellagra with eruption, 19 with scrotal lesions. Of these 4 had lesions only of the scrotum. Goldberger was able to find scrotal lesions with similar frequency in South Carolina and a special investigation supported his opinion that the lesions were frequently the first dermal sign

of pellagra. However the great frequency of the lesions among the 11 volunteers remained a source of puzzlement in view of the general opinion that such lesions were not common and Goldberger and Wheeler considered that special circumstances in the diet or environment of their volunteers may have played a part. The lesions themselves suggested their identity with pellagra since they were sharply delimited, dry, scaly and symmetrical.

TABLE 32
Frequency of Certain Cardinal Symptoms of Pellagra

| SYMPTOM                     | FREQUENCY |  |  |  |
|-----------------------------|-----------|--|--|--|
|                             | per cent  |  |  |  |
| Loss of weight and strength |           |  |  |  |
| Dermatitis                  | 96        |  |  |  |
| Glossitis                   | 82        |  |  |  |
| Tachycardia                 | 76        |  |  |  |
| Stomatitis                  | 66        |  |  |  |
| Peripheral neuritis         | 62        |  |  |  |
| Previous attacks            | 62        |  |  |  |
| Anemia                      | 60        |  |  |  |
| Diarrhea                    | 58        |  |  |  |
| Vomiting                    | 56        |  |  |  |
| Mental symptoms             |           |  |  |  |
| Achylia gastrica            |           |  |  |  |
| Other diseases              | 30        |  |  |  |

Based on 50 cases of severe endemic pellagra. Spies, T. D., Chinn, A. B., and McLester, J. B., J. A. M. A., 108: 853, 1937.

An investigation of the frequency of scrotal lesions confirms the view that, as judged by the clinical reports, frequency is most variable. It is possible they have frequently been overlooked since they occur on the posterior portions of the scrotum. But it may be that they represent a special deficiency. This is somewhat substantiated by such reports as those of Fitzgerald and Landor and Pallister, described in more detail elsewhere, in which similar scrotal lesions, but which itched severely, were found as the only dermal manifestation of an obviously deficient diet or associated with signs of riboflavin deficiency and nervous disorders, but without the usual skin lesions of pellagra. Spies, however, believes the scrotal lesions part of pellagra.

The mental symptoms of pellagra are manifold and range from insomnia to psychosis. In the mildest cases and in the prodromal period these symptoms are frequently considered to be neurasthenic and McLester, in studying the histories of hospital cases found that a considerable number had been in the institution the year before they were recognized to be suffering from pellagra, and were then diagnosed as neurasthenia. The symptoms complained of are insomnia, anxiety, vertigo, burning sensations, fatigue, palpitation, numbness, headache, distractability and headache. Conduct may be normal but the patient feels unable to work. More severe cases become melancholic and depressed. Lethargy and stupor are common. Occasionally hallucinations and confusion are seen. The Russian literature speaks of severe cases with fever, delirium and death as "pellagra typhoid."

In describing the early mental disturbances in pellagra Frostig and Spies emphasized the breakdown in personality. Previously brave and robust men become weary and apprehensive. They expect accidents and are pessimistic. Of the other early nervous symptoms of the disease headache resembling migraine is common. It occurs suddenly in the forehead and temples accompanied by scintillating scotomata.

Nervous manifestations include paraesthesias, tremors, muscular cramps, girdle sensations, pain in the neck, burning sensations in the eyes. Pressure pains in pelvis and pain on pressure over the spine occur. A common complaint is that the mouth tastes salty. The Italians sometimes speak of the disease as "Salso." Tremors of the hands and tongue are very common in severe cases and the gait may be paralytic

or spastic. Many of these are manifestations of beriberi rather than pellagra but are frequently associated with pellagra.

Circulatory system changes do not seem to be due to pellagra. Weiss and Wilkins conclude that the changes which are seen are due to thiamin deficiency. The heart in uncomplicated pellagra is small, the blood pressure tends to be low, both systolic and diastolic. Indeed these changes and certain other similarities to Addison's disease have led to the suggestion that pellagra was a chronic disease of the chromaffin and vegetative nervous system.

Mild cases of pellagra in children and cases without dermatitis are exceptionally difficult to identify. The children are undersized and underweight, have little ability to concentrate and progress poorly in school. Sore tongue, indigestion, vomiting and constipation are common symptoms.

More than half of all severe cases of pellagra are anemic. In most cases this is macrocytic and hyperchromic in type but frequently refractory to liver therapy. In a group of pellagrins reported by Spies and Chinn, most of which gave a history of alcoholism, 63 per cent were anemic. Of these, three-fourths had a color index above 1. Spies and Chinn consider that three factors may operate in pellagra to produce anemia, gastric dysfunction, iron or other deficiencies and hepatic damage causing failure to store the anti-anemia factor. The experiment of Sydenstricker and associates, already mentioned, in which extracts of a pellagrin's liver were found capable of inducing reticulocytosis in Addisonian anemia indicates the latter is not constantly true of pellagra but of course anemia is not constant either. Presumably anemia is not directly due to nicotinic acid deficiency but to a complication of pellagra.

# The spinal fluid is not conspicuously abnormal in pellagra.

# Natural History of the Disease

The natural history of the disease is characteristic and important. Most cases develop in late Winter or Spring, be-

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come more and more severe for two or three months and then slowly improve. Such an attack may never be repeated and the patient may progress to complete cure or vague nervous symptoms may remain permanently. Many more cases recur each Spring for a number of years. With each attack the patient becomes weaker and more emaciated until death occurs, in the average case after five years.

In a few cases the original attack becomes rapidly more severe and gastric and nervous symptoms more prominent. These are the acute cases and death is not infrequent during the first attack.

# Pellagra in Infants

Considerable interest attaches to the cases of pellagra in infants. That such occur has been known since the original report by Strambio, in 1794. Synder investigated the matter in 1912 and was able to report a case in a 2 months' old infant. The mother died of pellagra 2 weeks before the infant developed the rash but the midwife reported that the infant had been emaciated and sickly at birth and had had a profuse, foulsmelling diarrhea since the age of one week. Voegtlin and Harries, who cite these cases, report a remarkable instance in which the infant developed the classical signs of pellagra although the mother remained free of symptoms. Indeed neither the mother nor father had ever had the disease. The infant's earliest symptom was a sore mouth, first noted at 5 months. The following Spring diarrhea appeared and the following month (June) erythema of the face, hands and legs. The diagnosis was confirmed by physicians thoroughly familiar with the disease and the skin and mouth lesions were considered typical. Throughout the illness the infant was breast fed. These reports are much like those of beriberi in sucklings in which the mother is usually, but not always, simultaneously suffering from the disease. Pregnancy, and lactation in particular, seem definitely to predispose to pellagra. The incidence of the disease is very high at such periods. In Voegtlin

and Harries' case the mother's milk was analysed and found to be adequate in fats, protein, carbohydrate and minerals, results similar to analyses of the milk of beriberi mothers.

No discussion of pellagra in infancy and childhood would be complete without reference to the view of Goldberger that the disease in our own country is predominantly one of early life, between the ages of 2 and 15 years. Goldberger believed that the hospital records are misleading on this point because of the relative mildness of the disease during childhood.

# Atypical Pellagra

Atypical forms of nicotinic acid deficiency mainly fall in one of two groups, nervous disorders and combined deficiency disease. The former group is largely a recent development, the cases having been recognized only through their response to nicotinic acid.

The nervous and mental symptoms of pellagra have already been described. In districts where the disease is common physicians have naturally suspected similar nervous disturbances might be due to latent or early pellagra since, as has been mentioned, nervous symptoms are frequently seen to precede the systemic signs of the disease. Thus Cleckley, Sydenstricker and Geeslin report 19 patients without dermatitis or stomatitis but with nervous symptoms such as are frequently seen in pellagra. Many had sore tongues. All but 4 responded dramatically to nicotinic acid therapy. Musser's experience was quite similar. His patients were anemic, had sore tongues and porphyrinuria as well as nervous symptoms. Bogart observed a similar case the mental condition simulating catatonic schizophrenia. Cure was effected within the week.

Jolliffe, Bowman, Rosenblum and Fein announce that an "encephalopathic syndrome" occasionally seen in the psychiatric wards of Bellevue Hospital and which was heretofore almost invariably fatal responds to nicotinic acid. The syndrome occurs alone or in combination with pellagra or thiamin

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deficiency disease. It is characterized by clouding of consciousness, cogwheel rigidities of the extremities and uncontrollable grasping and sucking reflexes. Twenty-two cases were treated with large doses of nicotinic acid (1000 mgm. a day) plus 100 mgm. of sodium nicotinate given intravenously in dextrose solution and another 100 mgm. sodium nicotinate injected intramuscularly. Fifteen of the cases recovered. The authors suggested that this psychosis represents a result of a complete deficiency and develops before the dermal lesions have time to evolve.

Blankenhorn reports two cases, one resembled hemiplegia, the other diplegia. Both recovered under dietary treatment and the paralysis was ascribed to pellagra.

Pellagra sine pellagra accounts for many other cases of atypical pellagra. Manson-Bahr and Ransford, for example, describe a woman suffering from stomatitis, desquamation of the tongue and chronic diarrhea as such a case. They believe that the cutaneous lesions are less likely to occur in temperate climates.

Castellani proposes the term "dermoberiberi" or pellagroid beriberi to describe patients suffering simultaneously with pellagra and beriberi. The cases he has seen have shown the usual signs of beriberi in the extremities plus roughening of the skin with pigmentation and stomatitis including lesions in the angles of the mouth and keratosis of the hair follicles. The latter, according to the description, seem definitely to be lesions due to vitamin A deficiency and the angular stomatitis is probably the consequence of riboflavin deficiency. The rôle of pellagra in these cases is therefore not prominent.

That beriberi does occur in the presence of outspoken pellagra is evident from the report of Lewy, Himwich, Frostig and Spies. Prompt cure of the nervous symptoms followed treatment with thiamin. These authors believe that the neuropathy of pellagra is actually beriberi and not due to either nicotinic acid or riboflavin deficiency.

The cases described by Wilson as common in Soonchun and Fusan, China, responded to cod liver oil although they appear to have resembled pellagra very closely. The symptoms appeared in the Spring, in most cases recurring year after year. Dermatitis on hands and feet with desquamation and cracking, sore tongue and mouth, difficulty in eating and diarrhea were present. The tongue lesions were those of pellagra. It would appear that other dietary factors than those in cod liver oil must have accounted for the success of Wilson's treatment.

Moore reports that the natives of Nigeria frequently suffer from perleche, sore tongue and genitalia and retrobulbar neuritis. Yeast products are curative. There is little in this description to justify the diagnosis of pellagra. The perleche is now recognized as a feature of riboflavin deficiency and the lesions of the scrotum constitute a rather unusual expression of pellagra as has been pointed out elsewhere.

The cases described by Williams had the mouth lesions of pellagra plus corneal ulceration, nervous irritability and diarrhea. The spleen and liver became enlarged. The place for this syndrome among the deficiency diseases is uncertain. An enriched diet proved to be curative.

# Prognosis

The prognosis in severe cases of pellagra has previously been poor. Smith and Stevens collected the records of 520 patients treated by various physicians in California in the period 1928–1935. The mortality rate was 66 per cent. Among those patients having dementia, diarrhea and oral lesions the rate was 92 per cent. We believe these results to be quite representative.

However, even without nicotinic acid a much better prognosis may be assured by an expert, experienced hospital organization. This has been achieved by circumventing the difficulty in feeding these patients and by giving huge amounts of anti-pellagra foods, yeast and liver extract. Boggs and Padget

reporting the results in 102 cases of various degrees of severity, reported a mortality rate of 19.5 per cent. Before Goldberger's work only 26 per cent of a similar group handled in the same hospital recovered. In Voegtlin, Neill and Hunter's very complete studies along similar lines efficient dietary control of the disease among one hundred subjects was conclusively demonstrated. Shortly before the use of nicotinic acid mortality rates as low as 5 per cent were frequently reported. Spies, for example, said the mortality rate in the Lakeside Hospital in Cleveland had been reduced from 54 to 5 per cent by a regimen combining a high calorie diet with yeast, cereal embryo, or liver extract administered parenterally.

Nicotinic acid has further improved these results. Spies, Grant, Stone and McLester followed 321 clinically active cases. Hospitalization was necessary in only 30 whereas previous to the new therapy many more would have required hospital care. Not a single patient died of uncomplicated pellagra. The three deaths which did occur were due to other causes and occurred when the pellagra was improving or had

been cured.

### Treatment

Nicotinic acid functions as a specific in pellagra but our knowledge of its administration is still incomplete. One thing which seems well established is that the requirement of different patients varies widely. Dosage must be greater during an attack than as a preventive during a remission; it must be greater in severe than mild cases. The requirement varies with the season in persons on a marginal intake and is increased by exercise, infections and the character of the diet. Size and age are factors in establishing the requirement of individual cases. The prophylactic dose of nicotinic acid in depleted individuals varies from 50 to 1000 mgm. a day (Spies, Grant, Stone and McLester).

Acute cases and cases in relapse are best controlled by re-

peated doses, 50 mgm. ten times in the day (Spies, Grant, Stone and McLester). Large doses should be continued even in the event of toxic manifestations referred to below. One gram daily has been given without harm although most cases may be equally well controlled with much less. Fouts, Helmer, Lepkovsky and Jukes treated 3 cases with 0.5 gram daily and 1 with 1 gram. They considered these doses to be near the maximum. Ruffin and Smith have used 1.5 mgm. per kilo and found it satisfactory. A large number of patients were observed. This would seem to be near the minimal level of effective medication although the preventive and curative intake in black tongue disease is said to be slightly less.

Dosage has been determined so far both on therapeutic effectiveness and unfavorable reactions. The latter were studied by Sebrell and Butler. Sensations of heat and tingling in the skin occur with large doses. These symptoms occur within 10 minutes of swallowing the dose and persist for 10 to 20 minutes. The peripheral blood vessels are dilated and the blood pressure falls briefly. The skin temperature is increased, circumoral pallor occurs. Often gastro-intestinal hypermotility and even nausea and vomiting follow. Sebrell and Butler determined the dosage level responsible for these symptoms by prolonged tests in adult women. The drug was given orally in 10, 30 and 50 mgm. amounts. Women on the 50 mgm. doses first showed symptoms on the 12th, 16th and 27th day of administration. The symptoms regularly recurred with each dose thereafter and divided doses produced identical although milder reactions. One woman receiving 30 mgm. daily had a reaction on the 32nd day and at times thereafter. None of the women receiving 10 mgm. daily showed the symptoms referred to throughout the test, a period of 3 months. However, others have induced the reaction with 10 and 12 mgm. given intravenously. Evidently

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the route of administration plays a part. Sebrell and Butler state that reactions are to be expected in some patients receiving 30 mgm. by mouth and in most receiving 50 mgm. Present indications are that these symptoms, while disagreeable, are not dangerous. Toxicity studies in mice and dogs show the fatal doses of nicotinic acid to be very large indeed. Presumably, pellagrins are less prone to these symptoms than normal individuals.

In common with all of the other forms of treating pellagra, nicotinic acid is most effective in acute cases. Cleckley, Sydenstricker and Geeslin have emphasized the remarkable effect of such treatment in stuporous cases. The glossitis and acute skin changes respond promptly also. Constitutional and digestive improvement have been frequently noted as extraordinary. The chronic lesions of pellagra respond slowly and the ultimate effect on nervous disorders associated with pellagra is presumably not marked. Whether this represents the usual ineffectiveness of replacement therapy after organic change is well established is not known. Many cases present nervous disturbances due to vitamin B<sub>1</sub> deficiency. This may be treated by supplements of thiamin, the use of which is discussed in another chapter. In the same way the presence of signs of ariboflavinosis, scurvy or other deficiencies may be specifically treated and in any case these vitamins should be provided by supplying an enriched diet. A formula containing 3000 to 4000 calories of which milk is the chief constituent but which also contains meat juices, broths and 5 to 15 grams of a good dried yeast is very useful. As the soreness of the mouth and the gastric symptoms disappear solid food can be given.

Nicotinic acid may be given intramuscularly or intravenously if vomiting or the disturbed condition of the patient make oral administration ineffectual. It has been suggested that the excretion of the drug may afford means of regulating dosage.

Liver extract in large amounts is a valuable therapy in pellagra and probably supplies all of the factors commonly lacking.

Itching, moist skin lesions may be treated topically with potassium permanganate solution (1:5,000). This is recom-

mended by Spies, Chinn and McLester.

Nicotinic acid may prove useful in other conditions. Bing and Broager report that it exerts a considerable effect on sprue which is interesting in view of the points of similarity between sprue and pellagra (cystic colitis, anemia, etc.). In most other conditions in which it has so far been tested it has been found ineffective. It does not influence nutritional cytopenia in the monkey (Day, Langston and Darby) or Addisonian anemia (Spies et al. and Hansen-Pruss). It is said to be useful in irradiation sickness.

The prevention of pellagra is not a problem of nicotinic acid medication. Pellagra occurs where meat and dairy produce are not eaten in reasonable amounts, on farms with neglected kitchen gardens. The ill health due to pellagra would be but partly alleviated by supplying nicotinic acid, other deficiencies would remain. The problem is one of education and economic reform, or, as Drummond said: "of £.s.p."

# Laboratory Aids in the Diagnosis of Pellagra

The most extensively used and simplest laboratory examination is a test for urinary porphyrins which are present in even mild cases of pellagra. Spies, Sasaki and Cross consider an increased excretion an integral part of pellagra. Spies, Cooper and Blankenhorn identified the porphyrins as coproporphyrin I and III. Normal amounts appear following treatment with nicotinic acid (Spies, Bean and Stone). The technique for this test is described in the Appendix. A further clinical study of its use will be found in the article by Beckh, Ellinger and Spies. Watson denies that the pigments are porphyrins.

A simple test for urinary nicotinic acid has been described by

Vilter, Spies and Mathews. The results were read in a photometer. No color develops from the extract of urine of pellagrins or prepellagrins while normal individuals give a positive result. This method will also be found in the Appendix.

Mainzer states that pellagrins are extremely sensitive to insulin. The subcutaneous injection of 5 units was followed by a severe hypoglycemia which persisted for an entire day. Tscherkes, Litvack and Korovitzky report that pellagrins do not react at all to adrenalin. The pulse rate and blood pressure remained unchanged. This would be in sharp contrast to the behavior of cases of beriberi in whom adrenalin exaggerates the circulatory symptoms to a severe degree.

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### CHAPTER XV

# THE NATURE AND FUNCTIONS OF VITAMIN C

In 1757, James Lind published his classic "Treatise on Scurvy," the first clear account of the disease. Lind established the efficacy of lemon juice for its prevention and cure. In 1804, Sir Gilbert Blaine secured regulations enforcing supply of lemon juice to the sailors of the British Navy and in 1865, similar regulations were adopted for the mercantile marine. Funk in his first review of possible avitaminoses suggested that scurvy might be a vitamin deficiency disease but Holst and Fröhlich (1907) initiated modern research for this vitamin by showing that scurvy could be experimentally produced in guinea pigs.

The characteristic of the vitamin that proved the best clue to its nature was its instability. This was shown by the work of Zilva in England, by Vedder and by King in America and by Bezssonoff in France. In their attempts to isolate the vitamin from lemon juice or cabbage juice it became increasingly evident that oxidation rapidly destroyed the potency of the vitamin.

This was further confirmed by studies of the methods of preserving antiscorbutic foodstuffs. The commercial canning process (Eddy and Kohman, 1924–5) was found to owe its protective action against loss of vitamin C potency to control of oxidation. In 1922, Zilva showed that decitrated lemon juice lost 80 per cent of its potency in one-half hour if made N/20 alkaline and exposed to air at room temperature. No loss of potency occurred if air was excluded. This was confirmed by Kennedy in Sherman's laboratory in 1926.

These and many other similar studies proved that vitamin C (the antiscorbutic factor) is an easily oxidized compound and one whose oxidation is notably reduced by the maintenance of an acid reaction. For reviews of these studies see King.

Progress toward isolation of the vitamin was delayed from 1916 to 1920 by the infection theory of scurvy origin. 1916, Jackson and Moore recovered from guinea pigs made scorbutic by a diet of oats and milk, a diplococcus which they suggested might be the etiological factor. Since oats and milk were known to provide a complete diet for rats Jackson's results seemed to exclude diet as a causative factor.

The following year McCollum and Pitz confirmed the production of scurvy in guinea pigs by feeding diets of oats and milk and supported Jackson's theory. McCollum has described their attitude at the time in these words: "They found it difficult to believe that the disease could be due to the lack of a specific substance, for milk alone suffices as the sole food for all young mammals during a critical period of their lives."

Examination of their oats and milk fed guinea pigs showed the caecum distended with impacted feces. They therefore felt that this also confirmed the infection theory.

Shortly after, however, Chick and Hume and Cohen and Mendel produced evidence that reconfirmed the probable dietary origin of the disease. Chick and Hume showed that milk was a far poorer protective against scurvy than had been assumed and Cohen and Mendel by feeding a superior diet were able to produce scurvy in a guinea pig without developing impacted feces or producing caecal lesions.

A few years later Parsons furnished the final explanation of McCollum's inconsistent results. Parsons found that while oats and milk constituted a protective diet for rats this was due, not to the vitamin content of milk but to the complete immunity of the rat to scurvy. Parsons found that the livers of rats reared on a diet deficient of the antiscorbutic factor contained significant amounts of this factor and that rats, unlike guinea pigs and man, synthesize their needs of the vitamin. This was confirmed by further studies of Parsons and Hutton and by Lepkovsky and Nelson.

The false trail was therefore abandoned and search for the antiscorbutic substance resumed.

A specially effective aid to this search was found in significant contributions made by Tillmans and Hirsch. These chemists, at Frankfurt, Germany, had occasion to distinguish between fresh and stale and between true and artificial fruit juices. They found that distinction could be made by using an oxidation-reduction indicator known as phenol-indo-phenol. Fresh juice gave a strong reaction with the reagent, stale juices a lesser reaction. Artificial juices did not affect the indicator.

Zilva showed also that antiscorbutic juices bleach phenolindo-phenol. Zilva and his associates found that they could determine the reducing capacity of antiscorbutic potent substances by means of the indicator but that the results did not always parallel estimation of vitamin C by animal tests. From these comparisons Zilva reached the conclusion that:

Vitamin C itself did not reduce indophenol, but that the decolorization of the indicator was due to a reducing substance closely associated with the active principle, which tended to prevent oxidation.

To this view of Zilva's, Tillmans took exception and contended that it was the vitamin itself which bleached the indicator. In advancing this view he relied on studies that produced evidence that the reduction of the indicator was due to vitamin C itself, that the indicator measured the concentration of the vitamin and its physiological potency, and that the vitamin might be hexuronic acid. He held that the oxidation of the vitamin was reversible and that in the first stage of oxidation the vitamin was more prone to destruction by further oxidation than in its original reduced form.

Though Tillmans did not know of it at the time, Szent-

Györgyi had already demonstrated the reversible oxidation of hexuronic acid. The further resemblance of the vitamin to hexuronic acid developed a little later.

In 1932, Waugh and King precipitated from lemon juice an actively antiscorbutic substance which they were able to isolate in crystalline form. They reported that it appeared identical with the hexuronic acid Szent-Györgyi had recovered from adrenal cortex, oranges and cabbage. Waugh and King share the credit for the crucial work that identified vitamin C as a hexuronic acid.

Szent-Györgyi's part in this development may be summarized as follows: In 1928, he isolated from adrenal cortex a highly reducing hexose derivative, hexuronic acid. He noted at the time that the compound decolorized the phenol-indophenol reagent and this led him to its possible identity with the reducing substance postulated by Zilva. In 1932, the year in which Waugh and King reported their results, Svirbely and Szent-Györgyi furnished further evidence of the identity of hexuronic acid and vitamin C.

The structure of hexuronic acid was established by Haworth and Hirst and collaborators and in the same year, Reichstein, Grüssner and Oppenauer synthesized it.

The term hexuronic acid has been superseded by the name ascorbic, now universally adopted to designate the pure vitamin. The structure of this compound has been shown in Chapter II and the most commonly occurring form is what is known as l-ascorbic acid. Certain therapeutic preparations have used the term "cevitamic acid."

The outstanding characteristic of ascorbic acid is its ability to act as a reducing agent. As has been shown, when it oxidizes the process proceeds in two steps; the first step (lascorbic to dehydro-ascorbic) being reversible. As oxidation proceeds beyond the dehydro-ascorbic stage the substance is physiologically inactivated and ultimately breaks down to oxalic and threonic acids (see fig. 9).

In vivo or in vitro dehydroascorbic acid can be reduced to l-ascorbic acid by reagents such as H<sub>2</sub>S in acid solution, by cysteine, glutathione, and the fixed SH groups associated with the proteins. In such reductions the rate is markedly dependent upon the relative concentrations and on the pH of the solutions.

From figure 10 it is evident that titration with phenolindo-phenol indicator gives us only the amount of l-ascorbic acid present. If the solutions contain some dehydro-ascorbic acid we will not detect it by this titration. If, however, after determining the l-ascorbic acid by titration we treat the solution with H<sub>2</sub>S in acid solution and then titrate again, the difference will be the amount of the reversibly oxidizable dehydro-ascorbic acid. Such procedure is necessary in food assays to be sure we get the full potency of these products when ingested for the body can utilize the dehydro form as well as the l form.

In body fluids such as blood, urine, cerebrospinal fluid, the ascorbic acid appears to be almost wholly in the form of the l-ascorbic acid (Borsook; Farmer and Abt). Hence in titrations of these fluids with the indicator we can take the l-ascorbic acid found as representing probably the total antiscorbutic content of these fluids.

In urine titrations, however, there may be another source of error in the presence of substances beside ascorbic acid with a reducing action. pH control eliminates some of these non-ascorbic substances. According to Evelyn the amount of these interfering substances may be as high as 90 per cent of the total reducing power of urine though the amounts in blood and cerebrospinal fluid are small. Evelyn has shown that the rate of reduction by l-ascorbic acid is much faster than the rate of these interfering substances and such rate can be utilized to secure accurate estimate of the l-ascorbic present. Five seconds will usually suffice to titrate all the l-ascorbic present. (See Part II Chapter I for test methods.)

Certain strains of bacteria have been proven capable of

destroying l-ascorbic acid. Recoveries of test doses in urine and feces do not therefore necessarily represent differences between ingested and excreted amounts; some of the vitamin may be actually destroyed in the body by bacteria.

#### CHEMICAL AND PHYSICAL PROPERTIES

Crystals of pure l-ascorbic acid in the dry state are stable to air and light for long periods of time. In water solution, however, aerobic oxidation takes place, accelerated by heat, light, and the presence of flavins. Certain ions, notably copper but including iron, mercury, silver, manganese, ferricyanide, hydrogen peroxide, iodine, phosphotungstate, nitrate also accelerate oxidative destruction of the vitamin.

There has been described a specific ascorbic acid oxidase occurring in certain plant juices but King states that this has been found to be a copper-protein complex in which copper is the catalytic agent. Since copper is not specific for ascorbic acid oxidation the name should not be continued.

From the practical viewpoint we are interested in stability of the vitamin in order to determine dosage and requirement and to know how to treat foods in order to conserve the vitamin.

Eddy and Kohman showed in an extensive series of studies that the commercial process of canning produces very little destruction of vitamin C due to the protection against oxidation. There is apparently more danger from the blanching process than from the processing after the food is in the can. Fellers has studied the effect of freezing on vitamin C content and reaches the conclusion that at the temperatures used vitamin C is not much affected.

That the commerical canning of tomato juice results in a little destruction of C has been shown by a series of tests at the Connecticut Agricultural Experiment Station. They examined 30 different brands of canned tomato juice and found them to range from 13 to 32 mgm. ascorbic acid per 100 grams

of juice with an average of 19 mgm. (380 International units). Fresh pressed tomato juice varies according to various assays from 250 to 600 International units per 100 grams, with the

TABLE 33
Some Effects of Storage and Cooking on Vitamin C Potency
(After Tressler et al.)

| FOODSTUFF       | INTERNA-<br>TIONAL<br>UNITS PER<br>100 GRAMS<br>FRESH, RAW<br>PRODUCT | PER CENT LOSS<br>POTENCY AT ROOM<br>TEMPERATURE | PER CENT LOSS BY COOKING  |
|-----------------|---|---|---------------------------|
| Asparagus       | 343   | 20% in 2 days                                   |                           |
| Broccoli        | 2702  | 47% in 2 days                                   |                           |
| Cabbage         | 500-1200  | 20% in 19 days                                  | 66% in 12 minutes boiling |
| Carrots         | 88-128  |   | 44% in 15 minutes boiling |
| Chard           | 760   |   | 60% in 10 minutes boiling |
| Corn sweet      | 163   |   | 12% in boiling            |
| Lima beans      | 480   | 37% in 7 days                                   |                           |
| Peas, green     | 520   | 10% in 2 days                                   | 55% in 15 minutes boiling |
| Potatoes, white | 252   |   | 6% by boiling             |
|                 |   |   | 19% by mashing            |
|                 |   |   | 30% by frying             |
| Snap beans      |   | 35% in 2 days                                   |                           |
| Turnip greens   |   |   | 83% in 45 minutes boiling |

#### Apple Behavior

Apple sauce cooked 18 minutes undergoes 40% C destruction. Apple pies produce 80% destruction of vitamin C.

Relation of duration of heating to C destruction in apple sauce:

| 0 minute          | 0% destruction  |
|-------------------|-----------------|
| 4 minutes         | 30% destruction |
| 8 minutes         | 36% destruction |
| 12 minutes        | 37% destruction |
| 18 minutes (done) | 40% destruction |

average around 400 units. Evidently then, commercial canning conserves well the factor present in the fresh fruit.

Storage of fruits and vegetables at ordinary temperatures may, however, result in serious loss of this factor and cooking operations can produce serious losses.

Tressler and associates have made studies of these operations on quite a range of fruits and vegetables and some of

their findings are given in table 33.

The results shown in table 33 not only emphasize the difference between fresh, raw products and the cooked forms but also that "so-called" fresh fruits and vegetables may be far below the potency they had when harvested by the time they reach our kitchens and tables.

Synthetic vitamin C is today available in powder and tablet form. The crystals are readily soluble in water and have a melting point at 192°C. They have a specific rotation of +24 in water solution and of +48 in alcohol solution. They have a maximum absorption at 260 m  $\mu$  in water solution and 263 m  $\mu$  in alcohol solution.

The International unit of vitamin C is the equivalent of 0.05 mgm. of pure l-ascorbic acid. The International unit is approximately  $\frac{1}{10}$  of the Sherman LaMer unit first used in

this country.

The relation of vitamin C to clinical and anatomical effects is discussed in detail in the following chapters.

### VITAMIN C AND HEMORRHAGE

In experimental animals and in man an outstanding result of C deficiency is hemorrhage. In experimental animals such as the guinea pig the earliest symptoms are hemorrhages around the joints followed by bleeding gums and these symptoms are duplicated in human scurvy.

Capillary fragility appears to be the precursor to this bleeding and one function of vitamin C is undoubtedly to maintain capillary resistance against leakage. For the bleedings of scurvy vitamin C is specific. There are, however, other types of bleedings such as purpura for which vitamin C appears to be ineffective. It is therefore not the sole factor concerned with the control of hemorrhage.

Vitamin C dosage has been found helpful in combatting the

bleeding of gums and intestinal hemorrhage in certain types of colitis and hematemesis; also in preventing postoperative hemorrhages.

The relation of vitamin C to hemorrhagic diathesis and the relation of measurements of capillary resistance to dietary inadequacy of vitamin C are discussed in more detail in Chapter XVI.

#### VITAMIN C AND ANEMIA

It has been contended that vitamin C affects other blood conditions than hemorrhage, namely certain forms of anemia. Mettier and associates suggest that it may have a specific effect on red cell formation when there has been a chronic inadequacy of the vitamin. Abt and Farmer, however, incline to the belief that such anemias as occur in combination with C deficiency are more often the result of iron deficiency than dysfunction of the red cell producing tissues.

Stephens and Hawley produced evidence to show that ordinarily leucocytes are much richer in vitamin C than the blood plasma or red cells.

#### VITAMIN C AND THE TEETH

Vitamin C deficiency definitely affects the functioning of the odontoblasts in the formation of dentine. This is discussed under anatomical manifestations of vitamin C deficiency. Hanke has claimed that it is a factor in the prevention of gingivitis and also of dental caries. Boyle and associates studied its relation to periodontoclasia (pyorrhea) of a systemic type and concluded that in this type vitamin C deficiency is a factor. They describe two types of pyorrhea: One a local inflammatory disease and the other a systemic process causing atrophy of the alveolar bone. It is the latter type that they consider related to vitamin C deficiency.

Vitamin C deficiency may be one factor in prevention of

dental caries but present research indicates that it is not the primary or sole factor involved.

### VITAMIN C AND DETOXIFICATION

In 1935, King and Menten reported that when guinea pigs are given C-deficient or C-low diets they pass through a "prescorbutic" stage before actual symptoms of scurvy appear. In this stage they become more sensitive to injections of diphtheria toxin; dextrose tolerance is also lowered (Sigal and King).

King states that arteries, teeth, and adrenals are particularly sensitive to toxin injury in low vitamin C conditions. Bacterial toxins can reduce the adrenal content of vitamin C by 50 to 85 per cent.

In the Annual Review of Biochemistry, 1939, King cites a group of researches to support earlier observations as to the value of increased vitamin C in certain types of infection, notably with hemolytic streptococci, tuberculosis, rheumatic fever, peptic ulcer, and artificial fever (see Chapter XXIII).

#### VITAMIN C AND IMMUNITY

In 1935, Sulzberger and Oser noted a change in immunologic response of the skin toward neoarsphenamine when ascorbic acid was administered in large doses. Their observations were confirmed by Cormia.

Others have reported that there is relation between adequate vitamin C and immunologic response. Ecker and his coworkers have shown a close relationship between vitamin C deficiency and inactivation of guinea-pig blood complement. The reversible oxidation reduction behavior of complement appears dependent on the ascorbic acid content of the plasma and the normal activity of complement is contingent on its being in the reduced state. It is suggested that ascorbic acid is the principal substance operating to keep the complement in the reduced state.

### VITAMIN C IN RELATION TO ENZYMES AND HORMONES

In his review of ascorbic acid in 1936, King cites various reports supporting the view that vitamin C may influence the action of certain enzymes. But in 1938, he says:

At the present time it is impossible to indicate with certainty any specific relationships between vitamin C and the enzymes in animal tissues so far as normal physiologic processes are concerned; but many papers record activating and inhibiting effects on enzymes in vitro and it is not unlikely that some of the observed effects will prove to be physiologically significant.

Some of the enzymes that have been said to be affected by vitamin C are cathepsin, papain, amylase, arginase, catalase, urease, tyrosinase, and nuclease.

We know that l-ascorbic acid is an oxidizing substance capable of transport of hydrogen but again according to King satisfactory evidence is lacking of its functioning as a respiratory catalyst in animal tissues. Animal tissues depleted of C fail to show decreased respiration activity and when ascorbate is added to such tissues no increase in oxygen uptake occurs.

### VITAMIN C AND HORMONES

A relation between vitamin C supply and certain hormones is suggested by a number of observers. Demole and Ippen attributed to vitamin C an anti-thyreotoxic action. Pfleger and Scholl claim that saturation of diabetics with vitamin C intensifies the action of insulin so that carbohydrate metabolism can be regulated with smaller doses of insulin. There also appears to be a relation between vitamin C and adrenal function.

It is claimed that adrenalin decreases vitamin C excretion (Ohta); that glycogen formation in the liver is increased by giving adrenalin and vitamin C simultaneously (Imai); that C deficiency reduces adrenalin content of the adrenals and increases blood adrenalin (Doby and Weisinger), to cite only a few of the recent papers bearing on this relationship.

Addison's disease, skin pigmentation caused by changes in the suprarenal capsules and neighboring sympathetic plexuses is claimed by Wilkinson and Ashford to be associated with vitamin C deficiency and this view is supported by Hoff. Abt and Farmer report that in two cases of Addison's disease the daily administration of 450 mgm. ascorbic acid alone caused considerable depigmentation after 6 to 10 weeks, but failed to improve other symptoms. Their results are in agreement, with those of Siewe.

#### SUMMARY

It is obvious from perusal of the literature that much still remains to be done in the case of many of these postulates of vitamin C function. It is necessary to separate results obtained from malnutrition causes and to determine those in which vitamin C plays a specific rôle.

The Council of Pharmacy of the American Medical Association permits definite claims for vitamin C value only in the case of scurvy. They state that dental caries, pyorrhea, certain gum infections, anorexia, anemia, undernutrition and infection alone are not in themselves sufficient indication of ascorbic acid deficiency to permit the claim of benefit by ascorbic acid dosage, unless it is definitely stated that C will benefit only when such conditions are consequences of deficiency, suboptimal supply or imperfect assimilation of the vitamin.

They further state that "unless more convincing evidence is present than is now available, no claim referable to the antiinfective effect of ascorbic acid will be recognized."

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#### CHAPTER XVI

#### SCURVY

Hippocrates and Pliny both wrote of scurvy and Straby described cases of the disease in the armies of Caesar Germanicus and Aelius Gallus. Scurvy follows armies. It ravaged the troops of Napoleon in Egypt. During the Civil War 46,910 cases were reported in the Union Army with 771 deaths directly due to scurvy. An army post, at Council Bluffs, Iowa in 1821 had half its force invalided with scurvy of which 30 per cent died. Two important outbreaks have occurred in this century. This disease was still common in Russia at the turn of the century. In a number of years the incidence was from 2.9 to 5.3 per 10,000. This was ascribed to the backward vegetable and fruit culture. In the World War epidemics were common both in prisons and in the field. German troops had 23 epidemics on the Eastern front and 1 on the Western. Epidemics customarily occur in March and April in the temperate zones. The incidence increases rapidly, the curve reaching a peak in 4 to 6 weeks. Many historical references to scurvy were given by Hess in his monograph. Vogel gives a complete account of scurvy outbreaks on sailing vessels. It is interesting, in this regard, to note that the early expeditions to America were not only the cause of hundreds of cases of the disease but also led to a greater degree of control since these same mariners introduced potatoes to Europe (Sherman).

#### ETIOLOGY

Scurvy, as is well known, is due to a dietary deficiency of ascorbic acid, or vitamin C. But secondary etiological factors are quite as important and interesting in regard to scurvy as

to beriberi and pellagra. They doubtless are of critical importance in individual cases and may throw light on important functions and relationships. As is true of the other deficiency diseases these observations have been anticipated in the clinical literature.

The opinion of pediatricians twenty years ago may be gathered from two quotations. In 1917 Hess wrote: "Infantile scurvy is not, however, a simple dietary diease. diet is at fault in allowing the intestinal bacteria to elaborate toxins. It is doubtful whether the toxin is always the same and therefore whether strictly, from an etiologic viewpoint, this disorder should be regarded as an entity." In one of his last contributions to the subject he stated that he was still in accord with this view. Brennemann, in 1923, expressed the opinion: "Scurvy occurs only in children with a predisposition to the disease—and the individual factor may play a larger part than we now know." These views were based on observations of discrepancies between diet and the occurrence of scurvy. On the same intake not all individuals developed the disease. Scurvy sometimes appeared and disappeared although the diet was not changed. On the same diet one group of patients developed scurvy and another neuritis (Darling).

Is there any evidence that scurvy is more complicated than the general opinion holds? Is there reason to believe that these earlier observations were due to phenomena we now neglect and not simply to the cruder methods of study then available?

A discrepancy seems to exist between the calculated requirement and the occurrence of scurvy among certain groups. Stefansson has pointed out what a tremendous gulf this is. By the calculations of nutritionists primitive peoples should all be invalided by scurvy, he writes, whereas actually they are robust and healthy. He concludes that one of several errors exists in our present conceptions. Too much reliance has been placed on guinea pig experiments which are not ap-

plicable to man, the estimated requirements are 2 to 10 times too great, the ability of man to utilize and extract vitamin C from foodstuffs has been underestimated, the effect of cooking overestimated or there exists in animal tissues a component other than ascorbic acid which is capable of preventing scurvy. Possibly man can adapt to a low vitamin intake.

In guinea pig experiments reported in 1931 (Dalldorf) it was pointed out that animals placed on a vitamin C free diet developed capillary fragility but that this did not develop regularly but was subject to a transient reversal during the early stages of the depletion. In other words processes of adjustment or compensation were probably present. This view has since been confirmed. Giroud speaks of adaptation to explain the interesting observation that on a depletion diet the organ vitamin stores fall and then rise slowly so that they are greater at the end of the first month than at the 15th day. Wachholder had a similar experience. On a C free diet the stored vitamin was rapidly lost during the first 3 days (this period corresponds to the initial rapid fall in capillary resistance). From the 3rd week on the values of the intestine, lung and heart remain practically constant until death, while the liver, skeletal muscle and plasma show a transient increase. The capacity of various tissues to oxidize vitamin C and to reduce dehydro-ascorbic acid undergoes marked variations, being increased shortly before symptoms of scurvy appear and much reduced when the symptoms are fully developed. Wachholder observed the same mechanism in human cases. A deficiency of 300 to 500 mgm. is well counterbalanced. Only a deficit greater than 1000 mgm. produces symptoms. Baucke's studies of the oxygen consumption seems to indicate the same mechanism. While consumption is greatly diminished during the initial depletion period it increases for a time to greater than normal values shortly before the onset of symptoms.

Mouriquand has also written of this matter. In a recent study by Mouriquand, Edel, Dauvergne and Lavaud it is pointed out that guinea pigs, when they begin to lose weight show a remission of symptoms. Symptoms could be retarded and minimized by withholding water. On a regimen designed to limit the fluid intake guinea pigs survived for from 30 to 40 days on a C free diet. This may be due to reduced rate of glomerular filtration. The authors state that macroscopic evidence of scurvy was lacking at post mortem examination.

Whatever the mechanisms are which cause one individual to differ from another in ascorbic acid requirement or utilization they have a very practical significance as any group of cases will show. A typical example of this individualism was reported by Elmby and Warburg. Of 29 cases of mild scurvy (hemorrhagic diathesis plus low blood plasma vitamin values) 26 responded within 10 days to 300 mgm, ascorbic acid given orally. In all 26 the symptoms disappeared and the plasma value became normal. The other 3 showed no improvement in either respect. They were therefore given 300 mgm. parenterally on each of ten days and still failed to respond. Treatment was thereupon changed to the juice of 10 lemons daily. Under this regimen the blood values promptly rose and the symptoms disappeared. Elmby and Warburg suggested that another factor in the lemon juice was essential, in these particular cases, to utilization. Whatever the explanation the experience is not an unusual one.

Possibly the value of the diet in other factors modifies the vitamin C requirement. In the discussion of the incubation period of scurvy which follows reference is made to the recent reports in which individuals on a varied and ample basal diet survived for many months without ascorbic acid and without signs of scurvy. The epidemics of the disease have without exception been characterized by diets deficient in many factors other than vitamin C alone. These observations have led certain persons to suggest that man is capable of synthesizing ascorbic acid to a certain degree or that he can utilize small

amounts more efficiently if his intake is small. It would seem more in harmony with natural laws if the difference between species in respect to vitamin C, in which rats are immune and man and guinea pigs susceptible, were relative rather than absolute.

However these are purely speculative. Enough has been said to illustrate the problems which still exist. While they are not important in a consideration of ascorbic acid nutrition as it applies to the population as a group they are doubtless of considerable significance in understanding the erratic occurrence of the spontaneous cases of the disease and therefore of particular interest to physicians.

# THE ANATOMIC CONSEQUENCES OF VITAMIN C DEFICIENCY IN EXPERIMENTAL ANIMALS

The morbid effects of vitamin C deficiency are best studied in experimental animals where complete dietary control permits us to examine the lesions at all stages of the disease. We have therefore chosen to describe the pathology of experimental scurvy before we discuss the lesions in human cases. In both cases the lesions are, for all practical purposes, the same and it is interesting to recall that the commonly accepted explanation of the pathogenesis of scurvy, while established in controlled, experimental material, has only confirmed the theory of Aschoff and Koch which was based on human cases of the disease.

Aschoff and Koch characterised scurvy as a condition in which defective intercellular materials were formed. Wolbach and Howe substantiated this theory by demonstrating that in complete deprivation of vitamin C certain skeletal tissues formed a fluid material rather than their natural products, dentine or bone. The physical character of the fluid may be demonstrated by administering the vitamin which causes it to rapidly solidify or gel. The vitamin is therefore

an essential ingredient in certain intercellular materials. While this explanation is not generally accepted as yet it is in

conformity with our own experience.

The alternative explanation of the scorbutic lesion is that deficiency produces an atrophy of the skeletal tissue cells. This view was warmly defended by Höjer both on the basis of the minute structure of the lesions and on other observations among which were the decreased oxygen consumption, reduced gastric acidity, diminished adrenalin content of adrenal glands, retarded hematopoesis, decreased opsonic index and resistance to infection. To proponents of the "jellation" theory these are ascribed to secondary changes. The phenomena of complete deficiency and repair have not been satisfactorily explained on the basis of cellular atrophy.

The most recent contribution to the pathogenesis of scurvy is the work of Ham and Elliott who also object to the "jellation" theory and conceive of the scorbutic process as an atrophy. They found no histological evidence that either excess fluid was present during scurvy or that the rate of repair was more rapid than occurs normally in the healing of fractures. It is very likely that the issue cannot be settled by histological means. The reasons given by Ham and Elliott do not seem conclusive. A point which seems to favor the acellular character of the phenomena of acute scurvy is the generally accepted evidence that the formation of collagen procedes without the immediate intervention of cells. This view was expressed by Hertzler who said: "My researches have convinced me that the cell is not primarily the active agent, but that the initial processes are chemical and are identical with those of blood coagulation, the cell playing an entirely secondary role." Baitsell also believed he had observed collagen formation in fibrin clots in the absence of cells. Since ascorbic acid is of cardinal importance in the formation of collagen this evidence would seem to be further substantiation of the view that the essential phenomena of complete

deprivation are intercellular. Most investigators who have taken exception to this view have been dealing with partial degrees of deficiency.

Other techniques may be necessary to settle this problem. von Jeney and Törö, who grew fibroblasts in tissue cultures, report that ascorbic acid controls the formation of fibrils in the ground substance. More satisfactory conditions might be secured if collagen formation were studied free from cells. Laue patterns indicate that collagen is the result of crystallization in a sol. Such experiments are therefore possible and might well yield a decisive answer.

Experimental scurvy has been extensively studied by Glasunow who characterized it as a loss of the ability of differentiation by the mesenchyme. Studies of the healing of wounds, as reported by Mazoue and Randoin fully describe the effect of depletion on the formation of collagen and also reveal that the histiocytic and giant cell response to experimental granulomata is interfered with.

The guinea pig is the animal of choice in studies of scurvy, being very susceptible to deficiency of the vitamin. Rats, swine, fowl and calves are evidently immune. Many studies of the effect of vitamin C deficiency in the rat have been published.

"Rat scurvy" was first reported many years ago by Shipley, McCollum and Simmonds. The diagnosis was not proven. However Kollath, some years later, believed he had produced scurvy in rats by feeding a B deficient diet in which peanut oil was substituted for the cottonseed oil. One of his rats was diagnosed by Schmorl as a case of scurvy but the report is not given and the question has remained unsettled. An explanation of this curious state of affairs may lie in the observations of Muselin, Tully, Longnecker and King on the effect of inanition, various lipids, etc., on the excretion of vitamin C by the rat. Vedder and Rosenberg also report that large doses of a vitamin A rich oil caused scurvy like symptoms in rats and

that 0.5 mgm. of ascorbic acid protected the animals "almost fully." The possibility of producing scurvy in rats, fully immune to simple deprivation, is a very interesting one and might prove a useful tool in studying morbid mechanisms capable of producing scurvy. However these observations do not establish scurvy in the rat and until clear cut histologic evidence is submitted the problem can not be settled. The same is true of the other animals occasionally reported as scorbutic. Hjärre and Lilleengen found waxy degeneration in the muscles of young calves. This lesion is said to be relatively common during late winter and spring and in years of poor harvest in Stockholm, Germany and France. They claimed to have observed scorbutic lesions in the bones and teeth of such animals and to have produced similar lesions in very young calves. It may well be, therefore, that immunity and susceptibility to scurvy are relative rather than absolute.

It will be remembered that much earlier in the study of the avitaminoses (1918) Harden and Zilva found rats grew better if ascorbic acid was supplied and that Drummond found their litters were larger. At that time Drummond wrote of the rat's requirement: "they are sufficiently well marked to dispel any idea that there exists a fundamental difference in the nutritive requirement of the two types of animals" (rats and guinea pigs).

# Scurvy in the Guinea Pig

In sharp contrast to the rat the guinea pig rapidly develops severe and fatal scurvy. The first manifestations of the disease seem to be capillary fragility which is promptly followed by microscopic lesions in the teeth. Wasting develops rapidly within the second week and most animals die at the end of the third week of completely deficient diets. On partially deficient diets the animals live for months and develop the lesions we have long associated with scurvy in man.

The lesions pathognomonic of scurvy occur in the teeth and

long bones. In these structures the parenchymal cells, whether odontoblasts or osteoblasts form fluid instead of the normal substantial dentine or bone, or, in cases of partial deprivation they form defective materials which, while solid, are distinctly inferior. A definite law seems to govern the type of degradation product of these cells. On a moderately deficient diet the most highly specialised cells, the odontoblasts, form bone, but if the dietary deficiency is more pronounced they revert to simple connective tissue cells. The osteoblasts, less highly differentiated, form an inferior bone or collagen depending on the degree of deficiency.

The result of this fundamental change is that teeth and bones cease to grow, and become gradually more porotic and fragile. Thin bones are, accordingly, characteristic of both experimental and human scurvy. Other sequelae of these changes are weakening of the costo-chondral junctions and epiphyses, pathological fractures and separation of the periosteum at the sites of muscle insertions and hemorrhage beneath

the periosteum.

The fascia and ligaments are likewise weakened. Todd mentions this. "In certain unpublished studies of vitamin C deprivation in guinea pigs Dr. Milton B. Cohen and I have found such weakening of ligaments that it is difficult to skin the animal without tearing the ligaments of joints particularly those of the knee and cervical vertebral column. The head of the animal indeed is very apt to be torn from the vertebral column by that ordinary force necessary to draw the skin from the cranium."

## Skeletal Lesions

The first effect of deprivation in the bones is best observed at their growing ends. Here the active osteoblasts are seen, within the first weeks of the deficiency, to lose their round or oval shape and become fusiform. They migrate away from the trabeculae and are indistinguishable from fibroblasts, producing fibrils and even collagen if the deficiency be not complete. The proliferative zone of cartilage ceases to grow and is slowly resorbed becoming slender up to its end where it suddenly broadens into a thin junctional zone which is usually concave.

Adjoining this concave atrophic cartilage, in a developed scorbutic lesion, is a zone of fibrous tissue in which lie irregular, calcified and acellular fragments of the pre-existing trabeculae. This is the zone of the "Trümmerfeld" and "Gerüstmark," long associated with scurvy and responsible for the characteristic "lattice" in radiographs. In our experience the amount of fibrous tissue formed at the costo-chondral junctions is roughly proportional to the stresses present and is usually more pronounced in the lower than the upper ribs. If bone and cartilage are separated by strains the fibrous tissue response is magnified and forms a defective callus. These features indicate that the fibrous tissue is a defective response to normal stresses.

Associated with such fullblown lesions are hemorrhages and irregular masses of acidophilic material which Aschoff and Koch considered to be formed of fibrin. Schoedle, who appears to have been the first to describe this material, thought it was bone. This was also Höjer's view. Höjer observed the sequences leading to its canalization, demonstrated calcium in it both tinctorially and by ground sections. The material has two rather characteristic features, it lacks collagen and usually stains a brilliant red. Hart and Lessing, in their study of scurvy in monkeys, were also aware of this material and at first believed it to be fibrin. This view they surrendered when they were unable to find evidence of blood pigment, which should have been associated.

These unique and specific characteristics are almost immediately altered by the administration of vitamin C. The fibroblasts are promptly surrounded by a thin shell of osteoid material and resume their rounded form. Trabeculae rapidly

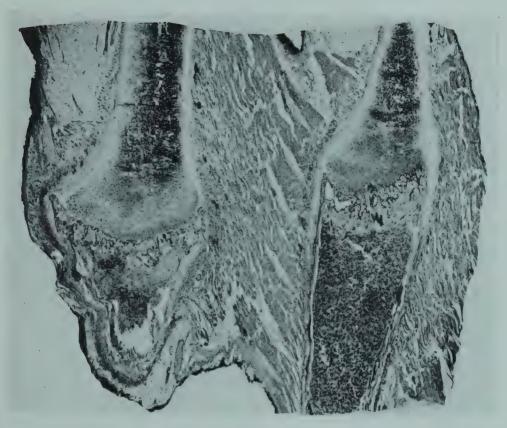


PLATE XXVIII. Late skeletal lesions in experimental scurvy. Two costochondral junctions showing the characteristic appearance of scurvy. The cartilage is thin and atrophic as well as the cortical bone. Between the two lies a zone which may be distinguished from the marrow by its lighter color. It is composed of connective tissue poor in collagen in which lie fragments of bone trabeculae. Osteoblasts are absent. Note also the reaction outside the periosteum. This is the lesion which is responsible for the characteristic picture in radiographs.



PLATE XXIX. Experimental scurvy. From the head of a femur in which fracture and separation have occurred. The head lies above, the diaphysis below and to the left. The callus which has formed is distinctly inferior in quality and is surrounded by "Gerüstmark" (as in the lower right hand corner.)

form, irregularly at first and gradually becoming more orthodox in appearance until nothing remains to indicate that scurvy has been present.

The sequences in the periosteum are essentially the same. If the fibrous layer is pulled free from the bone excessive fibrous tissue is formed. This is of an inferior quality although the cells themselves appear to be normal. Bone formation ceases and is replaced by connective tissue. The response to vitamin C is as rapid as that seen within the medulla.

Mouriguand, who has contributed many interesting observations to the literature of scurvy, and Dauvergne describe a special form of periostitis in guinea pigs which was produced by a careful adjustment of the vitamin C intake to a level which caused hemorrhages to appear only after 30 days of feeding. This spontaneously disappeared and 3 or 4 months later chronic changes occurred in the skeletal tissue. These consisted of a productive periostitis, ankylosis and stiffening of the hind legs. Pseudoparaplegia sometimes resulted as well as muscular contractions and signs of generalized rarefaction of the bones. The condition has many features seen in cases of chronic arthritis and "rheumatism" in man and is interesting in view of the frequently expressed opinion that some of these cases, especially those developing in the winter and spring, are due to a partial vitamin C starvation. disease in guinea pigs was found to be irreversible. Other causal factors may have been present. Some of the lesions had an inflammatory character and may have been the result of the activation of a latent infection by faulty nutrition.

Histologic evidence of defective collagen formation occurs not only in the bones and ligaments but elsewhere. It suffers universally (Höjer). In the earliest stages it is abnormal, irregular and uneven. Later its formation is completely arrested. In surgical wounds three things are evident. The vessel endothelium proliferates but no new vessels are formed; fibroblastic activity is normal; no collagen forms. Wounds

are therefore avascular and deficient in collagen. In the guinea pig skin, incisions do not heal (Wolbach and Howe). This is a typical and characteristic expression of the disease.

## The Lesions in the Teeth

The incisor teeth of a guinea pig are a more delicate criterion of the presence of scurvy than the bones. Within four or five days the odontoblasts may be seen to shorten and become separated from the dentine by a faintly staining fluid zone. If the deprivation is complete, and is maintained until the death of the animal, usually about three weeks, they revert to a spindle form and are indistinguishable from the connective tissue cells in the pulp of the tooth. Simultaneously the Tomes canals widen appreciably producing porosis of the dentine and the teeth cease to grow.

If the deficiency is partial and prolonged for several months the odontoblasts produce a substance resembling bone which gradually fills the pulp canal. Finally, as the incisor continues to grow slowly the tooth becomes more and more completely replaced by this bone-like material (osteo-dentine) which is then surrounded by a thin shell of pre-existing dentine. If cevitamic acid be then given the cells which lie within the osteoid matrix promptly elongate and enlarge and revert to their original appearance and function.

The tooth pulp becomes hyperemic, the vessels dilated, thin walled and engorged with blood and the pulp tissue atrophic. The dental lesions commence in the crown and extend rootward. They are so uniform that before the development of chemical methods of assay they could be satisfactorily used for the quantitative estimation of the degree of deficiency present and therefore the antiscorbutic value of the diet. This was possible either by judging them by their histologic structure, the Höjer score, or by observing their rate of growth (Dalldorf and Zall). The normal rate of growth of guinea pig incisors is 0.7 to 0.8 mm. per day. In complete vitamin C deficiency,

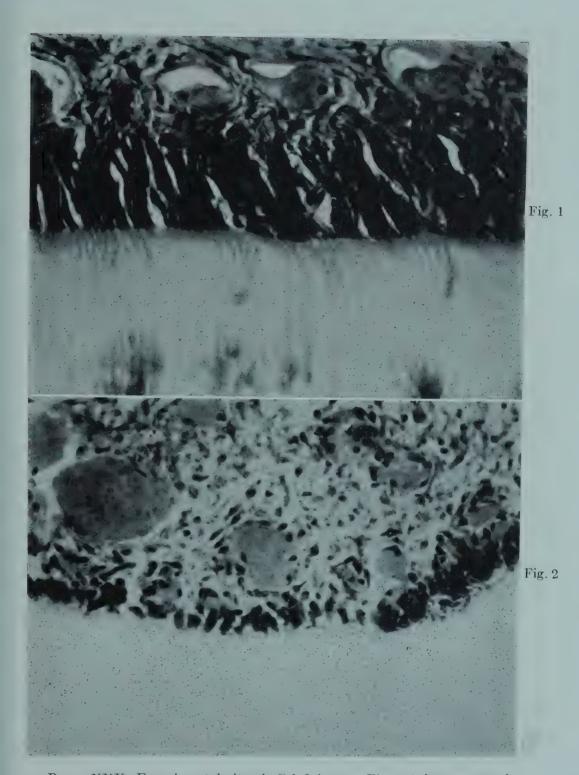


PLATE XXX. Experimental vitamin C deficiency. Figure 1 shows a normal junction of dentin and pulp. The odontoblasts are long and slender and stain deeply. Tomes canals may be seen extending in orderly, parallel fashion through the dentin. Figure 2 shows the effect of complete deprivation of vitamin C. Notice the atrophy of the odontoblasts and their separation from the dentin by an unstained zone. The vessels in the pulp are distended and thin-walled. (Reprinted by permission of the Journal of Experimental Medicine.)

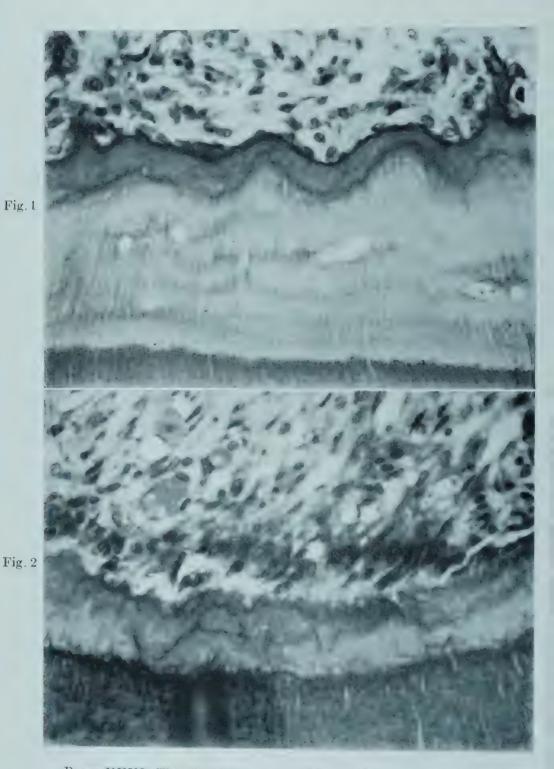


PLATE XXXI. The effect of complete deficiency of vitamin C for three weeks. Photographs of the teeth of guinea pigs. Figure 1 shows apparent disappearance of the odontoblasts. A zone of inferior dentin lies between pulp and pre-existing dentin. It contains no canals. The older dentin is rarefied, Tomes canals widened. Figure 2 shows an area in which odontoblasts may still be recognized as such although they fade into pulp structure.



PLATE XXXII. Experimental scurvy. Effects of recovery on the teeth of guinea pigs. Figure 1 shows the reappearance of odontoblasts and the formation, adjacent to them, of a zone of new dentin. Figure 2 shows more advanced repair. These photographs are of animals depleted as those in Plate XVII and then given vitamin. (Reprinted by permission of the Journal of Experimental Medicine.)

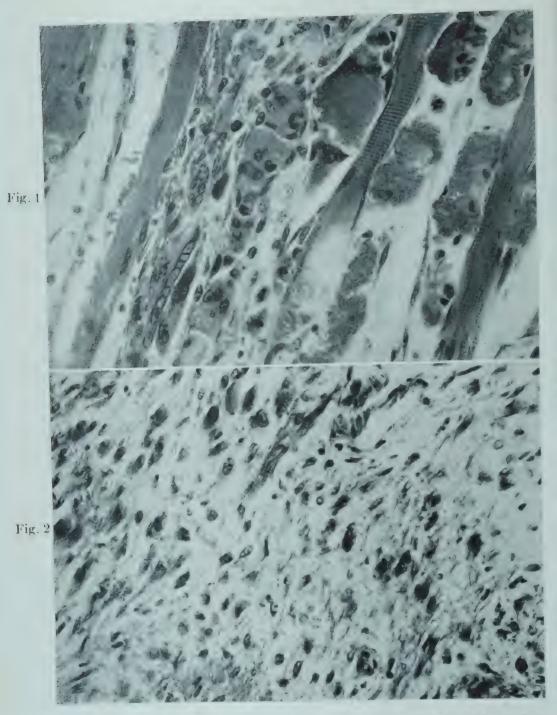


PLATE XXXIII. Experimental scurvy. Lesions in the skeletal muscles Figure 1 shows the early effects of deficiency in which fragmentation of the muscle fibers is associated with intense activity of the sarcolemma. The attempt at regeneration does not progress far and the late results (fig. 2) show loss of muscle fibers and replacement by collagen-poor connective tissue. Hyalinized fragments of muscle persist. Such lesions are seen in other deficiency disease but the repair is rather characteristic of scurvy.

after a short period of lag, growth ceases. On partially deficient diets the rate of growth is roughly proportional to the amount of vitamin C in the diet.

Harman and Kramer describe irregular, round elevations on the inferior surfaces of the mandibles in scorbutic guinea pigs. The bone was spongy in character and easily pierced with a needle. We have observed these lesions which are quite regularly present in protracted scurvy. They appear to be the consequence of rarefaction plus stress. The teeth are pressed into the mandible which is unable to resist and bulges into the periosteum. Höjer described these lesions in detail. They constitute a further example of the relation of stress to the lesions of scurvy.

Fish and Harris have studied the teeth of scorbutic guinea pigs and describe enamel defects which they believed might be related to caries. Their report is illustrated with several excellent longitudinal sections of incisors, both normal and scorbutic. Boyle has pointed out the defect in their observations, namely, that the degree of scurvy was sufficient to weaken the peridontal tissues and lead to dislocation and separation of the tooth and enamel organ. This explanation not only agrees with other known facts about the disease but is supported by Boyle's own results. As is shown in the discussion of avitaminosis A enamel defects and lesions of the enamel organ itself are the consequence of that deficiency. Indeed the enamel is disproportionally thick in scurvy due to atrophy of the dentine.

Boyle has described the effects of scurvy on the alveolar bone and states that vitamin C deficiency is the only nutritional disease, in his experience, which produces the characteristic features of systemic pyorrhea. A necessary distinction must be made between the local pyorrhea which develops about impacted food or from disuse. In cases of generalized pyorrhea a relative immunity to caries is quite common. After several months of a partial vitamin C deficient diet

Boyle's animals showed peridontal weakness and many teeth, but particularly the molars, became displaced. Boyle states that all of the patients of a large dental clinic whose blood plasma ascorbic acid was less than 0.5 mgm. per 100 cc. have shown similar gingival lesions which respond to specific therapy.

#### Lesions in the Vessels

By analogy it is believed that a similar inferior intercellular material is responsible for the hemorrhagic diathesis present in both human and experimental scurvy. Unfortunately no histologic evidence of such a change may be seen in the capillaries, but Wolbach has thoroughly demonstrated that while there is no lack of growth capacity in the capillary endothelial cells fully formed capillaries do not occur during complete deprivation. Furthermore the demonstration that the capillaries lose their fragility within a few hours of administering vitamin C suggests that something comparable to the results of treatment on the dental lesions also occurs in the vessels. Müller suggests the principle alteration is in the supporting mesenchyme of the smaller vessels and capillaries.

In the older literature mention is made of various vascular lesions in scurvy, fatty and hyalin degeneration and inflammatory processes which were considered responsible for the vascular weakness. Aschoff and Koch were unable to substantiate these results in their material and such lesions do not occur in experimental scurvy.

All of the manifestations of scurvy are greatly modified by the presence or absence of stress, and all of the lesions may be understood if this factor and the basic one of defective intercellular substances are appreciated. Stress determines the location of the hemorrhages. Protected vessels rarely rupture; stress augments the Gerüstmark and constitutes an essential element in the production of the muscle lesion (vide infra).

The hematopoetic tissue in the bone marrow is not char-

acteristic in scurvy. The capillaries and small veins are greatly dilated during the early stages of the disease and sometimes these dilated vessels simulate small hemorrhages. Hemorrhages occur also but these are usually near the epiphysis. Höjer agreed with Moore and Jackson that the larger vessels

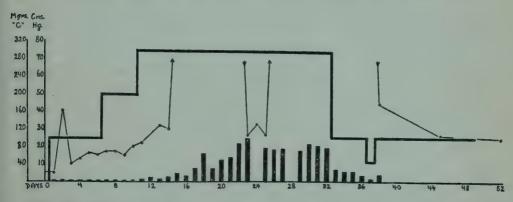


Fig. 21. Effect of cevitamic acid on capillary resistance and urine content of vitamin C in a severe case of scurvy. Mgs. 'C' represents the values of vitamin. The heavy line indicates the intake, the total excretion during the six hours following administration is shown by the solid columns. All of the vitamin was given parenterally. The thin line represents the capillary resistance in cms. Hg negative pressure as measured by the suction cup method.

Observe the prompt and extreme response of the capillaries the first day, the immediate recession and gradual increase thereafter. This phenomenon has frequently been observed by us. The arrows indicate that the capillary resistance exceeded the limits of the apparatus i.e., was greater than 65 cm. Hg. The drop in resistance on the 23d day has not been correlated with other features of the case. Observe that under the influence of massive doses of vitamin the capillary resistance was double what seems to be normal for the man. The time relationship between vitamin intake and capillary resistance is approximately the same during increasing and decreasing dosages.

The urine contained 0.4 mg. per cent and the blood less than 0.1 mg. per cent on admission. As measured by urinary excretion this man remained unsaturated for more than two weeks despite a total intake of 3.5 gms. of cevitamic acid. However his urine was always alkaline and the actual values may have been higher because of this effect. Symptomatic recovery commenced on the

fifth day and was complete on the tenth.

had inordinately thin walls. This was thought the result of atrophy of the collagen. Hess' view that the hyperemic marrow of rickets contrasts with the paucity of vessels in scurvy appears to be true only of the late stages of scurvy. In the early periods the marrow is hyperemic. Schmorl's theory of the pathogenesis of scurvy was that it was a direct result

of the anemia of the marrow. At that period vessel lesions were considered the fundamental disturbance in scurvy.

Lesions occur in the muscles in scurvy. Höjer felt that the myopathy had characteristic features, among which were pronounced necrosis with a tendency to calcification and the formation of giant cells which represent abortive attempts at regeneration. These cells have been generally interpreted as multiplying sarcolemma and their rate of growth may sometimes be so excessive that they suggest a myosarcoma. Excepting for the calcification, which has rarely occurred in our own material, the lesions are indistinguishable from those produced by other dietary deficiencies (Pappenheimer) or by other means (Forbus). Höjer ascribed the weakness which appears early in scurvy to the myopathy and described similar changes in the heart muscle to account for the cardiac weakness. found myocardial lesions in ten of thirteen animals but the most extensive lesions were in animals infected with tuberculosis. We have never seen cardiac lesions comparable to those described by Höjer. Fatty degeneration of the myocardium is common.

Scurvy produces atrophy and degeneration of the germinal epithelium as Medes has shown, and early destroys the ability to sire litters. In the female the oestrus rhythm is maintained in moderate deficiency but is suspended if the deficiency is severe enough to produce emaciation. The adrenals atrophy. This may be due to loss of cortical fat which is reduced in the terminal stages of scurvy. The reduction in cortical fat is related to the loss of vitamin C in the same tissue as Bessey, Menten and King demonstrated with the silver nitrate reaction. Atrophy of the lymphoid tissue of the lymph nodes and spleen is pronounced and some atrophy occurs in the salivary glands, kidneys, and liver. Hemorrhages and erosions of the stomach and intestinal tract have been reported by several investigators. They were noted by Holst and Frölich in the duodenum. More recently Smith and McConkey reported an

incidence of approximately 25 per cent most of which were in the first part of the duodenum. These lesions were erosions, usually covered with a small blood clot. Similar lesions have been reported in human cases.

Scorbutic guinea pigs frequently have premature litters. The young show retarded growth and many are dead. In cases of partial deficiency the young may apparently be born partially depleted of vitamin and quickly become scorbutic themselves unless the maternal milk is supplemented with vitamin C (Ingier). The pregnant animal is relatively immune to scurvy. Symptoms develop rapidly after parturition.

A moderate secondary anemia occurs in experimental scurvy and promptly responds to vitamin C. The blood changes have been studied by Meyer and McCormick. Höjer believed that the regeneration of both erythrocytes and granular leucocytes was retarded but that anemia or leucopenia were dependent on excessive destruction of the cells in which case the faulty hematopoiesis became apparent. Leucocytosis failed to develop in his scorbutic pigs when they were infected. The bone marrow is natural in appearance. Presnell found the coagulation time increased 54 per cent and the platelets reduced 36 per cent. The decrease in platelets is paralleled by the decrease in erythrocytes.

Mention is frequently made of edema in experimental scurvy. Moore and Jackson, whose work was very important in inaugurating the more recent study of the disease in guinea pigs, spoke of the edema about the joints and bones. The basal diets used were not complete. Doi has recently demonstrated diminished plasma proteins and increased water content in scurvy which appears to have been due to the deficiency itself. Höjer's view, expressed before Doi's study, was that edema was due to an associated deficiency. On a complete basal diet his animals were free of edema. Some of the fluid about the bones in scurvy may represent unjelled intercellular material.

The scorbutic guinea pig characteristically sits in a hunched position with drooping head, the so-called "tooth-ache position," has swollen and tender joints, and, if the disease is severe, is greatly emaciated. The guinea pig requirements of vitamin C are relatively large. According to Dann and Cowgill approximately 0.5 mgm. cevitamic acid is needed for each 100 grams body weight. However considerably larger amounts of antiscorbutic vitamin are required to prevent all of the manifestations of deficiency. Three mgs. of ascorbic acid are needed to insure natural tooth growth and nearly as much to prevent histologic lesions in the incisor teeth in pigs of 250-gram size. In the guinea pig as well as man there therefore appears to be a degree of vitamin C underfeeding in which distinct morbid effects are present without the clinical or grosser manifestations of scurvy.

Abercrombie described lesions in the thyroid gland in guinea pig scurvy. The follicles were irregular, their cells columnar; colloid was reduced and the interfollicular cells increased.

Giroud has been most active in studying scorbutic tissues by means of silver stains which he believes selectively color the ascorbic acid within the cells. There is still some uncertainty as to whether the method is specific for ascorbic acid but there can be no doubt that many of the phenomena of scurvy can be followed by this technique. Giroud's monograph should be consulted. These studies and somewhat similar ones by Glick and Biskind have related the site of storage of ascorbic acid to definite portions and cells of the tissues. The glands of internal secretion have been most thoroughly studied since these contain the largest amounts of the vitamin and relationship to function would seem especially close. Thus the zona fasciculata of the adrenal has been shown to have double the vitamin content of the medulla. In the pituitary gland the pars intermedia is especially rich in vitamin. Fluctuation in the ascorbic acid content of

luteum tissue has been correlated with its functional activity and parallels the concentration of progesterone.

Some mention is made later on of the nervous symptoms in human scurvy. Few observations have been made of the nervous system in the experimental disease. Meyer and McCormick reported peripheral degeneration and changes in the motor cells in the anterior horns. No functional disturbances have been observed.

In certain groups of guinea pigs fed the Sherman-LaMer diet fatty livers are common. This is not related directly to the degree of scurvy nor is it constant. Spellberg and Keeton ascribe it to the combined effect of ascorbic acid and an unidentified dietary factor.

#### MORBID ANATOMY OF HUMAN SCURVY

The significant lesions in human cases of scurvy are the hemorrhages and the skeletal defects.

The hemorrhages may take place in any organ and may vary from petechial size to huge extravasations. On the skin they are located about the hair follicles, sweat glands and skin lesions. They are common over bony prominences and in infants on the inner surfaces of the thighs where the diaper rubs. The deeper hemorrhages follow the fascial planes. Massive hemorrhages occasionally cause death in scurvy as in a recent case where a huge hemorrhage occurred within the pericardium.

The skeletal lesions are identical in their minute structure with those in the guinea pig and the commonest sites of the lesions are the costo-chondral junctions, the distal ends of the femurs, proximal ends of the tibias and femurs and the wrists. The distal end of the humerus and the proximal end of the ulna are usually spared. A feature of the bony lesions in man is the conical widening of the ends of the long bones. In severe cases chest deformities are seen which have been de-

scribed as a bayonet deformity when the dislocated diaphysis

pushed deeply into the Gerüstmark.

The muscle lesions are identical with those which occur in guinea pigs. They presumably occur only in severe cases. Höjer's view that they were responsible for the early weakness in scurvy is not supported by anatomical evidence that they actually occur at that time. The most extensive description of them in human material may be found in Mahé's monograph. Mahé pointed out the striking relationship to stress. Soldiers develop muscle lesions in the thighs and buttocks, artisans who work with arms and hands develop muscle lesions there, etc. The lesions are usually complicated by hemorrhages.

Lesions also occur in the teeth of man. Westin described eighteen cases, some after recovery and others with various degrees of scurvy. The lesions in the pulp include hyperemia and edema with atrophy, degeneration of the odontoblast layer with the formation of small cysts, destruction and calcification of the vessels, and necrosis and calcification of areas in the pulp. Osteoid tissue forms about the calcific masses and calcified vessels. The dentine undergoes porotic changes, with resorption along Tomes' canals which widen into spindle and round spaces. The formation of abnormal and irregularly canalized replacement dentine occurs. Similar changes occur in the cementum. Lesions are most common in the apical third of the tooth and at the bifurcation of the root canal. In acute cases fluid was seen about the odontoblasts.

Westin also demonstrated lesions in the teeth before the development of bone lesions. The parallelism between experimental and human scurvy is, therefore, complete in all studied features. Since Walkhoff's demonstration of congenital scurvy in the guinea pig with lesions of the teeth, we may anticipate that congenital dental lesions in man will some day be described.

Conspicuous changes occur in the mouth. The gums be-

come swollen and bleed easily, and rarefaction of the alveolar bone causes the teeth to loosen and fall out. The characteristics of the gingival lesions are a rapidly developing hyperplasia of the papillae with a tendency to spontaneous and intractable hemorrhage, disintegration of the epithelium, commencing on the papillae and followed by the development of granulation tissue and finally gangrene (Westin). Trauma obviously plays a large part in localizing these lesions since they do not occur at all unless teeth are present, and then at sites of greatest irritation and stress. Scorbutic lesions of the mouth are strictly limited to the gingiva in contrast to the mouth lesions of various factors of the vitamin B complex.

The skin lesions are petechiae which occur, characteristically, about the hair follicles. This is probably due to the rich plexus of small vessels there and the minute traumata they are exposed to by contact with the unyielding follicle. The keratotic changes so often associated have been present in certain epidemics and not in others. In Aschoff and Koch's cases, drawn from various nationalities in the Balkans the incidence was high among certain races and not in others. This we believe to have been due to differences in eating habits. Vitamin C deficiency must have been widespread but the diets varied in vitamin A values depending on the eating and cooking practices of the different nationalities. Two factors make it extremely doubtful that the lesions are scorbutic. They may or may not occur and they are by structure and incidence typical expressions of vitamin A deficiency. The lesions are described in Chapter VI.

Patients with scurvy are commonly pale and anemic looking, and frequently show slight edema about the ankles. In the older literature anasarca was frequently described. Hydropericardium and hydrothorax are often noted. The heart is frequently enlarged. Erdheim found enlargement in twenty-one of thirty-one cases. The enlargement of the heart is due to hypertrophy of the left ventricle. The stomach is

frequently congested or stippled with petechiae, and small duodenal ulcers are not uncommon.

Infantile scurvy is rare before the fifth month of life. Hill has described a case in a Chinese infant three weeks old. Jackson and Park have reported a typical case of scurvy in an infant twenty days old. Symptoms had been present from birth. The diagnosis was confirmed by autopsy.

# Scurvy and Senility

Aschoff and Koch were greatly impressed with the similarity of the scorbutic lesions in the skeleton to those in senility. The changes in cortical bone are difficult, if not impossible to distinguish. The distribution of the lesions is not the same in the two conditions but this may be due to differences in mode of life. In both conditions the bones are notably thin and rarefied, susceptible to fracture and defective in the ability to form callus once fracture has occurred. The suggestion seems a worthy one and would be included in the discussion of the significance of vitamin C in modern life were it not for the problem of pathogenesis it raises.

Westin interpreted the tooth lesions as similar to the atrophy of old age and said scurvy may be considered to hasten involution. In his cases the teeth showed the same resistance to caries that is seen in senility as well as the rarefaction common to advanced years. Fish and Harris pointed out that the osteodentin formed in scurvy is "normally" present in senile teeth and represents a scar about dentin which has died. Höjer considered the primary disturbance in scurvy to be atrophy of skeletal parenchymal cells, in the case of the teeth, the odontoblasts.

It is extremely doubtful that the problem can be resolved by histologic study alone. A comparison of the irregular dentin, or osteodentin produced in human teeth about carious cavities in well fed and scorbutic subjects might clarify the subject. The experimental studies seem to us to be much more

significant because of the uniformity of the subjects and the ready availability of all stages of the lesions. Wolbach and Howe's observations in complete scurvy are substantial evidence. It is difficult to correlate such a phenomenon with the decadence of senility. We are familiar with no other adequate explanation of their results than that, in the absence of vitamin C, the odontoblasts form an inferior, substitution material. That similar material (osteodentin) forms in man in a variety of conditions does not establish that in the case of scurvy it is not defective in quality. Indeed the extent of its formation in human teeth, in scurvy, suggests that it may be inferior and that, being so, larger amounts are required to afford the support and defense the rarefied tooth requires.

Possibly the rôle of vitamin C in the control of tissue respiration is the clue which will aid us in our understanding of senility.

# The Anatomical Diagnosis of Scurvy

The anatomical diagnosis of scurvy can be made from a gross examination of the tissues if the case has progressed to an advanced stage. In other cases the occurrence of unexplained hemorrhages or muscle degeneration requires the histologic examination of samples of the skeleton. In the costochondral junctions various stages of the typical scorbutic lesion will frequently be found. It is not necessary that it be fully developed. Experience rapidly recognizes a general fragility of the connective tissue fibers, a suggestive watery zone about the osteoblasts and a defective osteoid tissue formation which plainly indicate the presence of scurvy. It is often difficult to distinguish scurvy from other dystrophies of bone such as osteogenesis imperfecta. The distribution of the lesions and the presence of small hemorrhages are helpful in these cases. If suspicion exists at time of necropsy a specimen of the liver and adrenals should be taken for measurement of the ascorbic acid content.

### THE DIAGNOSIS AND TREATMENT OF SCURVY

The clinical recognition of scurvy is relatively simple if the morbid anatomy is appreciated. The cardinal symptoms are a hemorrhagic diathesis, weakness and signs and symptoms related to skeletal lesions and hemorrhagic gingivitis. In the present chapter the commonest symptoms will be described as well as the manifestations of partial ascorbic acid deficiency for while florid scurvy is at present a medical rarity mild forms of the disease are quite common.

#### Incubation Period

Older writings usually fix the interval between restricted diets and the onset of symptoms as between 4 to 8 weeks. Stevenson placed the incubation period at 6 weeks. But obviously this varies with the adequacy of the previous diet. Thus the usual sequence of affairs has been that the diet was of a low ascorbic acid value and then still more restricted because of the failure of the potato crop or the absence of stored vegetables. The time required to deplete a properly fed individual to the point of symptoms is much longer. Rietschel and Mensching and Rietschel and Schick report personal experiences on a vitamin C free diet. In the first report the subject (Dr. Mensching) lived for 100 days on a mixed dietary from which foods containing ascorbic acid were excluded or in the case of certain dishes precautions were taken to destroy the small amounts of vitamin which were probably present. Mensching's blood level of ascorbic acid fell from 0.7 mgm. per cent to 0.29 mgm. within 3 weeks. After 2 months it was only 0.17 mgm. and on the 100th day was 0.05 mgm. per cent. Despite this regimen no symptoms or signs of scurvy appeared.

In the second case Dr. Schick lived under similar conditions for 160 days. Depletion, as measured by the plasma values, developed at the same rate. The blood value remained at a level of 0.05 mgm. from the 116th to the 154th day. No

symptoms were noted although the gingival papillae were somewhat reddened and eroded but this was said to have been true before the experiment started. van Eekelen lived on a vitamin C free diet for 84 days without noticeable effects but Widenbauer, who fed a deficient diet to two idiot infants reported tenderness of the thighs and radiographic evidence of scurvy after 3 months. It therefore seems evident that the incubation period of scurvy can be a matter of months if the individual has previously been well supplied and does not suffer from predisposing causes. It is possible that these modern experiments differ so widely from the experience of older writers in part because the basal diet used in the experiments was complete in other respects whereas the epidemics of scurvy have occurred under conditions of general depletion. Reference has been made to this in the discussion of etiology.

Infantile scurvy is the commonest form seen in our own country. A helpful discussion of this subject has recently been contributed by Park and associates. In agreement with previous reports these authors found that swelling and tenderness of the thighs was the common symptom. "Pain and tenderness of the extremities or symptoms referable to pain such as disinclination to move, crying when handled, drawing up of the legs, 'rheumatism', gave the first evidence of the disease in 92 per cent of the cases." The pain was usually accompanied by irritability and fretfulness. Hess emphasized pallor and a worried expression as common features of scurvy during infancy.

The mouth lesions do not occur if the infant be edentulous but are present in 80 per cent of those with teeth (Park). This is a matter of considerable historical interest. In Barlow's treatise of fifty years ago this inconsistency was given careful consideration and Barlow's correct interpretation of the relationship of the teeth to scorbutic gingivitis was one of the major points in establishing the identical nature of adult

and infant scurvy. Barlow recalled that Sir James Paget had said that edentulous persons did not salivate from mercury and assumed that a similar mechanism occurred in scurvy. He also observed that infants with identical symptoms did or did not have swollen gums depending entirely on whether teeth had erupted or were on the point of erupting or the infant was toothless.

The onset of symptoms was usually abrupt in the 125 cases reported by Park and presumably appeared only after the skeletal lesions were well developed. Hemorrhages were infrequently observed but in most of their cases the observations were not planned to study this feature. Older writers have reported that a few red blood cells may usually be found in the urine during some stage of the disease.

Park's cases were largely in infants seven to nine months old. The depleted diet responsible for the scurvy was in most instances milk which had been both pasteurized and boiled. On such a diet symptoms appeared within two to nine months but lesions developed earlier. We will return to the significance of this observation in the discussion of subclinical scurvy.

Hess that that scurvy was frequently not recognized in places where it only occurred sporadically. It is often confused with rheumatism. One of us has recently seen a case of scurvy in an infant who was thought to have osteomyelitis of the femur. The epiphysis had separated from the diaphysis, the temperature was 103°, the leucocyte count was 14,000. Cultures at the operation were sterile and at autopsy the characteristic lesions of scurvy were found.

Clinical diagnosis of milder cases of scurvy is difficult and uncertain. Frölich has reported eight characteristics which he considers helpful. These are dystrophy, anorexia, anemia, occasional slight edema, cessation in gain of weight or loss of weight, susceptibility to infection, intestinal disturbances and now and then more pronounced symptoms such as hema-



PLATE XXXIV. Scurvy in an adult. Observe the petechial hemorrhages on both lower extremities and the swollen, brawny right leg and lower thigh.



 $P_{\rm LATE}$  XXXV. Scorbutic gingivitis. Swollen, boggy, hemorrhagic papillae with ulceration about the middle incisors.

turia. He considers that the diagnosis is never definite in such cases until the effect of the administration of the vitamin is noted.

Adult patients suffering from scurvy complain of weakness, pains in their legs, swollen, bloody gums and hemorrhages. Examination discloses petechiae, chiefly about the hair follicles of the lower extremities and sometimes brawny, tender thighs. All of these features are due to hemorrhage and it is interesting to recall that older writers considered a hemorrhagic diathesis as pathognomonic of scurvy. Recent work shows that this opinion is more justified than was thought a few years ago. The hemorrhagic diathesis is the essential manifestation of vitamin C deficiency, is responsible for most of the signs and symptoms of the disease and a diagnosis of scurvy cannot be made in the absence of a tendency to bleed.

Weakness is usually the first thing complained of by persons suffering from vitamin C depletion. Fatigue, palpitation and breathlessness are also common. The patients dislike to stand or walk and often affect a rather characteristic standing position with their legs slightly flexed. The complexion is pallid and dirty looking. Gingivitis occurs, followed by loosening of the teeth, a consequence of resorption of the alveolar bones and infections about the teeth and is accompanied by a foul breath. Other signs of scurvy are hematuria, bloody diarrhea, nasal hemorrhage or hematomas about the jaw or bones of the lower extremities.

The hemorrhages were considered as pathognomonic of the disease by older writers. They may occur in any organ and may cause confusing diagnoses. Thus hemorrhages in the lower right quadrant have been mistaken for appendicitis and hemorrhage in the transverse colon for a neoplasm. A diffuse, firm and infiltrating hemorrhage in the thigh was first considered to be a sarcoma. A pericardial hemorrhage caused death in a case described by Barton and Freeman.

Fever is usually present at some period of the illness and

complicating infections are extremely common. If death is not due to intercurrent disease it comes suddenly with syncope.

Under modern conditions few cases progress to the stage of prostration and diagnosis is more difficult. The most valuable diagnostic signs are again due to the hemorrhagic diathesis. Swollen, but not ulcerated gingiva, rheumatic pains and the most varied expressions of capillary weakness may be found or the latter may reveal itself in the form of protracted epistaxis and petechiae, without the mouth or muscle symptoms. The mouth lesions are absent or much delayed if the teeth are sound and clean and the sub-periosteal hemorrhages with their resultant rheumatic pains or painful masses do not occur unless the patient has been active, since they depend on stress.

The fever which often occurs during the course of both experimental and human scurvy has never been satisfactorily explained. Mouriquand, Pouzet, Schoen and Belly have recently described a case of scurvy in a girl two and one-half years old in which the fever was constant until orange juice was given. It then sank to normal only to reappear when the antiscorbutic was reduced. The original dose of three lemons and four oranges was resumed and the temperature became permanently normal.

Many writers have divided scurvy into different forms or stages. In animals, where the diet is rigidly controlled, this can be successfully done, as witness Höjer's classification. In human cases a natural division seems possible between manifest and latent scurvy, to which has recently been added subclinical scurvy. Older classifications of manifest scurvy seem of interest chiefly because they demonstrate that the severity of the case is expressed by the extent of the hemorrhagic diathesis. In the first phase of scurvy, according to French writers, slight bleeding of the gums and small hemorrhages about the hair follicles ("Piquete scorbutique") are found. In the second phase ecchymoses, especially about the lower leg and knee, are conspicuous. In the third phase

extensive hemorrhages from the mouth, nose, stomach and bowel and extensive skeletal hematomas develop. The tendency to bleed is therefore not only the salient clinical feature of scurvy but varies in degree with the severity of the particular case. In the mildest forms, the subclinical cases, a weakness of the smaller vessels exists without spontaneous rupture and can be demonstrated by measuring the capillary resistance to artificial stresses (the capillary resistance test). Aschoff and Koch spoke of the vascular changes as "angiodystrophy."

There are no essential differences between scurvy in infancy and adult life and in both cases varied manifestations of the tendency to bleed easily, pallor and rheumatic pains are the salient features. The confusion that formerly led to a separation of infantile scurvy (Moeller-Barlow disease) from the disease in adults was due to a combination of rickets and scurvy, often with other dietary deficiencies as well. E. Fraenkel's case of a seven year old boy with hemorrhages about the joints and skeletal lesions of rickets illustrates the difficulties of older writers. In adults, of course, scurvy is rarely complicated by lesions of the skeletal system other than those characteristic of the scurvy itself. The diagnosis must frequently be made between scurvy and rheumatism and between scurvy and blood dyscrasias. A satisfactory hematological examination usually suffices in the latter instance and the therapeutic test is of great assistance, especially since the isolation of the vitamin which has made the parenteral administration of large doses feasible.

A moderate degree of anemia is usually present in scurvy. This is of a microcytic or normocytic type and is distinguishable from other secondary anemias only by therapeutic test. Reticulocytosis and recovery follow the use of vitamin C while iron and liver extracts are ineffectual.

Proptosis is a late symptom of scurvy. In 1898 the American Pediatric Society reviewed 379 cases of scurvy. In 40

proptosis had occurred. The lesion appears suddenly as a result of hemorrhage either beneath the periosteum or into the orbital fat. The blood pigment sometimes moves forward to form a blue line along the lower orbital margin.

"Scurvy Sclerosis" is the term given the tender, brawny hematomata which may occur in the thigh in scurvy. We have seen this lesion in elderly patients on two occasions.

Of the cutaneous manifestations of scurvy, other than the perifollicular petechiae, are the keratosis suprafollicularis present in certain of Aschoff and Koch's cases, the scorbutic pemphigus of Lutton and the scleroderma like lesions described by Morawitz and Pfeiffer. Of these the most frequently mentioned is the keratosis suprafollicularis and this is almost certainly the result of a simultaneous vitamin A deficiency. Thus Mahé describes lesions he designates as lichen pilaris which are identical with those due to vitamin A deficiency: hard, elevated follicles with huge extruding masses of concretions giving the skin a pebbled appearance. The hairs were often broken off and stood like bristles or were lost entirely. Lind said the skin was usually soft but sometimes quite rough "like the skin of a snake." The epidemics observed by these early authorities were associated with other signs of vitamin A deficiency. Nightblindness was very common in Krebel's cases, so commonplace that its occurrence in scorbutics attracted little attention. Some early writers considered nightblindness a predisposing cause of scurvy and Hulme, writing of the epidemic of scurvy in the English fleet in 1761 spoke of nightblindness as the most important symptom. Under these circumstances vitamin A deficiency dermatosis could be expected to be present and the records show it was.

Ecker and Pillemer followed the complement titer in two classical cases of scurvy. Before treatment the titers of complement were very low, as was the blood ascorbic acid. Treatment caused a parallel rise in both until the blood ascorbic

acid passed the 1 mgm. per 100 cc. value after which no further increase in complement titer occurred. This corresponds to the blood level associated with maximum complement activity in guinea pig scurvy and the authors suggest that complement is a good biological index to scurvy. Very similar results have been reported by Chu and Chow who studied, in addition, cases of subclinical scurvy and found a correlation between blood ascorbic acid and complement in these cases also.

More recently hemolytic complement has been carefully followed in monkeys and guinea pigs by Chapman and Harris who found no diminution in complement throughout depletion or during severe and even fatal scurvy. The administration of ascorbic acid was likewise without influence. Whether this discrepancy is due to the methods used or differences in the basal ration is uncertain. It appears extremely doubtful from the observations of Chapman and Harris that a relationship between complement and vitamin C actually exists.

## Subclinical Scurvy

Mild, atypical cases of scurvy are much more frequent than clinically definite ones. They masquerade as rheumatism, gingivitis, purpura, hemophilia and osteomyelitis. They may be definitely diagnosed only by demonstrating depletion of ascorbic acid and cure by specific treatment. There usually is little question about the diagnosis if scurvy is considered.

But there are still more individuals who lack even these symptoms of scurvy and yet who prove to be depleted of vitamin. It is a natural feature of the avitaminoses, in contrast to the major infectious diseases, for example, that all degrees of deficiency, and therefore presumably of ill health may occur. For these conditions the term "subclinical" scurvy has been proposed.

This conception rests on rather substantial ground. Scurvy has been long recognized to have an asymptomatic stage which precedes the characteristic symptoms and, which is

more important, a degree of scurvy is recognized which is without definite symptoms although well developed and characteristic lesions may be present. The experience of Park and his associates is especially illuminating. In reporting 125 cases of scurvy in infants Park emphasizes the circumstances which made possible the recognition of most of these cases. Twenty years previously Dr. Martha Elliott inaugurated thorough radiographic and anatomic study of children dying from any cause, principally to determine the incidence and character or rickets. Out of this material, comprising 532 cases, 125 instances of scurvy appeared. In many cases diagnosis rested exclusively on histologic examination or on histologic and radiographic evidence. It was found that both of these methods might yield definite evidence of scurvy in the absence of symptoms of a recognizable kind and it was further discovered that even the anatomic diagnosis of the disease was frequently missed unless the pathologist was very alert to it. In other words the diagnosis of scurvy is difficult and frequently missed and the disease may exist in a well developed form without symptomatic clues to its presence. The full significance of this is not yet understood but modern studies have yielded engrossing results concerning the prevalence of such cases. The same is true of experimental scurvy. Eddy noted that lesions could be demonstrated in animals fed sufficient ascorbic acid to prevent symptoms and Dalldorf and Zall demonstrated that to insure normal growth of guinea pig incisors a much higher level of vitamin was required than was needed to prevent lesions. Zilva found that 10 times as much ascorbic acid was required to saturate a guinea pig than to prevent clinical scurvy. There is, indeed, complete agreement that such a condition of depletion exists and can be demonstrated at will. The issue is whether it is important.

Zilva concluded that it was not, that saturation did not represent optimal conditions. Szent-Györgyi on the other

hand states that clinical signs are late, premortal evidence of deficiency, that the subclinical state predisposes to infection and other diseases and has a great significance. The seemingly tremendous amount of vitamin necessary to saturate a guinea pig is not more than an animal living naturally in the tropics would consume. It is not fair to base requirements on the behavior of a caged animal. This is much the point of view taken by Giroud who believes a fair normal requirement would be the amount necessary to maintain organ concentration at those levels which non-susceptible animals have. Thus the rat adrenal quite constantly contains 1 mgm. or more ascorbic acid per gram tissue while the value in the guinea pig fluctuates greatly. Since the adrenal of an animal independent of dietary supply is 1 mgm., says Giroud, we may assume that this is the amount necessary for the proper function of that particular tissue.

It may be seen, therefore, that there are at least two conceptions of what is meant by subclinical scurvy, an asymptomatic form of deficiency and a mild, atypical form of symptomatic scurvy. To which might be added a third type; cases of vitamin C depletion or atypical scurvy associated with or due to other diseases. A good example of the latter is the situation which exists in cases of chronic peptic ulcer.

## Peptic Ulcer and Vitamin C

Schultzer studied patients with gastric ulcer who were placed on the usual therapeutic diets. One-third of the group had increased capillary fragility after sixteen days. In 70 per cent of these, treatment effected a return to normal. Many authors have confirmed this. Croft and Snorf found 15 or 18 patients with peptic ulcer had blood plasma ascorbic acid values below 0.4 mgm. per cent. Harris, Abbasy and Yudkin found the average excretion of such patients to be only 5.6 mgm. per day (approximately one-third normal). Portnoy and Wilkinson studied large groups of normal con-

trols, miscellaneous ward controls, patients with peptic ulcer and patients with hematemesis by various methods. The urinary excretion of the groups averaged, in order, 29, 17, 7 and 7 mgm. per day. The saturation test showed that from 2 to 8 grams of ascorbic acid were required by the last 2 groups. This considerable variation probably is due to the same factor which prevented 30 per cent of Schultzer's cases from responding to diet. The blood plasma of the peptic ulcer and hematemesis cases was at or very near the scorbutic level, 0.14 to 0.59 mgm, per cent. Nielsen had similar results. Lazarus found 10 of 12 cases of hematemesis unsaturated, 7 were extremely deficient. Ingalls and Warren examined 20 cases of peptic ulcer of which 18 were found to be depleted. The average blood plasma value was 0.19 mgm. per cent. Euler and Otto, Chamberlin and Perkin, and Bourne have all described similar results. Bourne used capillary tests as his criterion.

It would seem reasonable to conclude that patients with peptic ulcer are characteristically depleted of vitamin C. Yet there is little evidence that they have symptoms of scurvy, unless the associated bleeding is partly due to scurvy. Contrarily peptic ulcer is an uncommon complication of scurvy. Scorbutic animals frequently have small hemorrhages in the mucosa of the stomach but not ulcer. One is forced to conclude that either the dietary of ulcer patients is grossly deficient, their requirements abnormally high or their utilization imperfect. Do these low blood values have any significance in the handling of patients? Taffel and Harvey have measured the tensile strength of surgical wounds in guinea pigs on normal and deficient diets. The scorbutic animal has a stronger wound on the 4th post operative day but a weaker one on the 6th day. Even the partially depleted animals had demonstrably poor union on the 8th and 10th days, due in all likelihood to the defective collagen formation characteristic of scurvy. This possibility should be considered. An-

other application may prove to be the usefulness of vitamin C in the treatment of hematemesis. We have followed a considerable number of such cases and frequently observed hemorrhage cease after large doses of ascorbic acid. Replacement therapy might improve the status of these patients in other ways. Any efforts along such lines will require rather large amounts of ascorbic acid.

In like manner many cases of hemorrhage may be related to a stage of vitamin C depletion. Epistaxis is certainly one of the most common. We have followed a number of cases in which recurrent epistaxis, without obvious organic cause, was found associated with ascorbic acid depletion and responded immediately to specific, replacement therapy. Selected cases of menorrhagia and metrorrhagia behave similarly. Frequently cases which seem at first examination to be purpuras or other hemorrhagic diseases may be explained on a vitamin deficiency basis. But can we apply our knowledge of scurvy to still more unrelated conditions?

One of the lesions of scurvy is degeneration and fragmentation of the skeletal muscles, Zenker's degeneration. Study of older records shows how consistently it is present during scurvy. Mahé reviewed the older literature. The muscles turned to "currant jelly" and were sometimes so extensively diseased that the patient was unable to stand. The same degeneration occurs in experimental scurvy. Histologically it is identical with Zenker's degeneration as seen in typhoid fever. It would seem reasonable to inquire whether the degeneration in the muscles is due to typhoid fever per se or to a secondary form of vitamin C deficiency. The evidence is only suggestive but illustrates what may well be a profitable point of view. Thus 20 years ago typhoid fever was shown to produce a negative nitrogen balance. We now know that, in common with most other febrile disease, the ascorbic acid balance is also negative. The late stages of typhoid fever are complicated by hemorrhage. The intestinal bleeding is generally ascribed to the ulcers but oozing is also very common. Epistaxis is known to be a relatively common complication. Occurring in the late stages of the disease it often warns of impending death. Thus typhoid patients may be assumed to have depleted stores of vitamin C, inadequate intake (certainly in the days in which Zenker's degeneration was common), an unexplained hemorrhagic diathesis and degeneration of their muscles. To which might be added Beneke's interesting observation that all of the infants he examined post mortem during the war which died of sepsis had Zenker's degeneration. Since that time it has become relatively uncommon. The War period in Germany is known to have been a period of endemic subclinical scurvy as the papers of Meyer and Nassau show.

Other causes, including nutritional ones, are known to produce Zenker's degeneration. But the most likely to complicate typhoid fever seems to be scurvy. The response of typhoid patients to high calorie diets was proven by Coleman twenty years ago. Could not more be done for these people now if the balance of vitamins as well as nitrogen were considered? It is an interesting possibility. Other aspects of this problem are discussed in Chapter XXIII.

# Special Tests for Ascorbic Acid Deficiency

We are surfeited with tests for ascorbic acid deficiency but still hungry to know what deductions can safely be drawn from them. It is impossible to compare all the procedures which have been described. The present discussion will be limited to those with which we have had personal experience. The latter fall into one of three groups; tests of capillary fragility, of the blood or urine concentration of ascorbic acid or of the degree of saturation. (Technical instructions may be found in the appendix.)

The measurement of capillary fragility antedates the chemical tests by many years. Auspitz (1881) drew conclusions

from cupping patients with scurvy which seem to be the earliest references to this phenomenon. Hess performed many tests using a tourniquet to congest the vessels and determine whether they were fragile or not. Later Hecht devised a small glass cup connected with a variable negative pressure system and a manometer. Thus two methods exist for measuring the strength of the capillaries, one using positive and the other negative pressure. Both tests doubtless respond to the same conditions and essentially the same results have been secured with each. The reports of Wright et al. and Göthlin should be consulted for studies based on the tourniquet test.

What evidence exists that capillary fragility is a measure of scurvy? It is first of all generally accepted that the hemorrhagic diathesis is the primary or one of the primary, characteristics of the disease. But there are various other reasons.

- 1. Capillary fragility is present in most cases of manifest scurvy and its intensity is related to the degree of the scurvy.
- 2. It responds immediately to parenterally administered ascorbic acid and slowly to oral medication, as scurvy itself does (Dalldorf and Russell, Adant and others).
- 3. The seasonal fluctuation in capillary fragility parallels that of vitamin C intake and of serum complement (discussed elsewhere).
- 4. It is absent at birth when blood vitamin and stored vitamin are present in high concentrations and appears during the first year of life when the infant's ascorbic acid supply falls (Hoffman).
- 5. Capillary fragility is more common in bottle than breast fed infants. Children with vitamin C supplements have more resistant capillaries than comparable children without supplements (Roberts, Blair and Bailey). Fragility is a common characteristic of poorly fed children (Dalldorf).
- 6. Capillary fragility is present in infants with idiopathic hemorrhage, intestinal bleeding (Ratnoff), pachymeningitis

hemorrhagica interna and erythrocyturia minima (Hoffman). It has been prevalent to a pronounced degree when latent scurvy has been prevalent (Meyer).

7. Many of the conditions associated with capillary fragility which were formerly not believed related to scurvy are now known to be associated with vitamin C depletion. Among these are bottle feeding, infectious diseases (in particular, whooping cough, scarlet fever), and thyroid medication.

The weakness of the test is that certain other conditions which evidently are not related to scurvy also cause fragility. Thus Elliott's studies of thrombocytopenic purpura show that fragility is closely related to the course of that disease. Weld has demonstrated that ultraviolet irradiation alters the capillary resistance. Changes also occur from chemical and biological toxins, diurnally and between different parts of the body and even opposite sides of the body. It is not regularly related to blood plasma vitamin C (Abt, Farmer and Epstein), nor have all investigators found a relationship between saturation and resistance. However this is less important than a lack of relationship between scurvy and resistance which has not been proven to exist.

A thorough trial of the capillary test as a measure of vitamin C deficiency in groups of children has been reported by Roberts, Blair and Bailey. Their report is recommended both as being a thorough trial of the test and a good review of the experience of others. A distinct, statistically significant correlation was found between season, capillary resistance and ascorbic acid intake. The differences between the children on an institutional diet and those receiving supplements of vitamin C (bananas) are shown in table 34.

The virtue of the capillary test is that it is a measure of scurvy and capillary fragility due to vitamin C depletion, as identified by a test dose of vitamin C and followed by observations of the resistance is prima facia evidence of a pathological degree of depletion. This the chemical tests do only by

inference. There is no reason to believe that it is precise or uniform to any greater degree than other measurements of body function and much of the criticism of it has come from individuals who have looked for a degree of precision which the test lacks. The method of reading the results should reflect this limitation and the small differences between individuals overlooked in a search for distinctly abnormal, pathological responses.

Capillary resistance, blood and urine ascorbic acid, and saturation tests have all been studied in our laboratories. Typical results were reported by Sloan.

TABLE 34

| GROUP   | NO FRAGILITY PRESENT | FRAGILITY PRESENT |  |
|---------|----------------------|-------------------|--|
|         | per cent             | per cent          |  |
| Control | 32                   | 26                |  |
| Treated | 53                   | 5                 |  |

The blood ascorbic acid values for the same groups were:

| GROUP   | 1 MGM. PER CENT OR<br>MORE | LESS THAN 0.7 MGM. PER CENT |  |
|---------|----------------------------|-----------------------------|--|
|         | per cent                   | per cent                    |  |
| Control | 13                         | 64                          |  |
| Treated | 38                         | 26                          |  |

Of the single determinations the fasting blood level proved to be the best index to the patient's nutrition. The single test of urinary vitamin C is frequently very misleading. This may be improved by following Vauthey's suggestion of estimating the "basic ascorburia" (milligrams ascorbic acid per cubic centimeter urine of the excretion within one hour after the first matutinal micturition). Vauthey considered this to be a physiological constant. It would seem to be the best method of judging vitamin C balance by a simple urinary determination.

The most precise method of estimating the status of an

individual is by determining the blood curve following the injection of a standard test dose. This and other results are shown in figures 22–26 inclusive, based on Sloan's studies. The rate of excretion after a test dose is satisfactory in most cases. Originally the 24 hour excretion was measured.

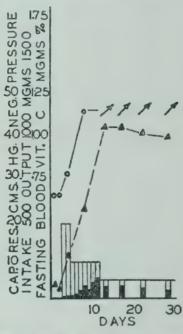


Fig. 22. Rate of recovery of normal blood concentration of vitamin C, capillary resistance, and urinary excretion of vitamin following oral administration of 400 mg. of vitamin C as orange juice. Also showing maintenance of normal values on 100 mg. of vitamin. The solid line represents the capillary resistance values, the broken line the concentration of vitamin in the blood plasma, the hollow columns the intake, and the solid columns the amount excreted in twenty-four hours. The patient suffered from hyperemesis gravidarum, and had epistaxis and hemorrhagic gums. According to the history her diet had been adequate until six weeks before treatment; the deficient diet was extremely restricted. (This and the following four figures are based on cases studied at Grasslands Hospital by Dr. Ruell A. Sloan. See J. Lab. & Clin. Med., 23: 1015, 1938. Reproduced by permission of the author.)

Equally reliable results may be secured by measuring the excretion during a 3 hour period.

Smith has suggested calling the capillary resistance test a measure of the physiologically indispensable intake, the maintenance of uniform excretion following saturation a

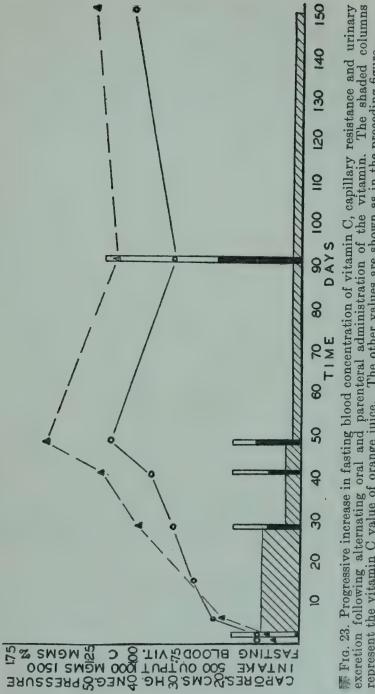


Fig. 23. Progressive increase in fasting blood concentration of vitamin C, capillary resistance and urinary excretion following alternating oral and parenteral administration of the vitamin. The shaded columns represent the vitamin C value of orange juice. The other values are shown as in the preceding figure.

test of adequacy, and the maintenance of saturation a measure of saturation or luxus consumption. This seems to accurately epitomize these procedures. The following correlations between such tests is based largely on our own experience.

Saturation. Best determined by blood curves following a test dose is regularly accompanied by a capillary resistance above 40 cm. Hg negative pressure as determined by the suction

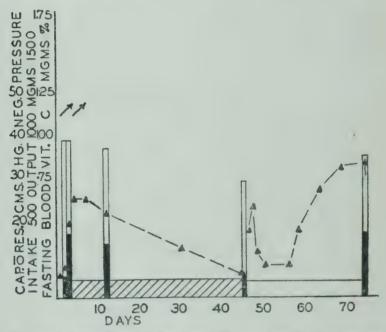


Fig. 24. Failure to maintain saturation and normal blood values of vitamin C on oral intake of 250 cc. of orange juice daily. Recovery on the same amount given parenterally (following the forty-fifth day). The patient suffered from peptic ulcer and had severe hemorrhages previous to treatment with vitamin C. Falsely negative capillary resistance test due to anemia.

cup technique or by a negative test by Göthlin's tourniquet method. The urinary excretion following a test dose is greater than 40 per cent within 3 hours of administration. The blood curve is elevated throughout the test period. The blood plasma values are almost invariably above 0.8 mgm. per 100 cc.

Partial Depletion shows intermediate values in blood curves,

urinary excretion and capillary resistance. In most cases the latter will be positive in the range of 15 to 35 cm. Hg negative pressure. The blood plasma values may vary from 0.2 mgm. per 100 cc. to nearly 1 mgm.

Depletion reveals prompt absorption of a test dose from the blood stream, the curve falling within two hours to subnormal values. Little or no vitamin C is excreted. The blood

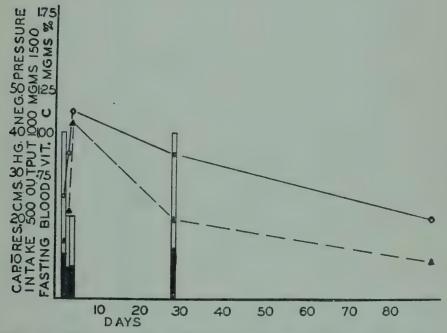


Fig. 25. The immediate response to large doses of vitamin C as shown by capillary resistance tests and blood concentration of vitamin and the subsequent fall in both values during a period in which the patient was maintained on a diet poor in vitamin C.

plasma values are less than 0.2 mgm. per 100 cc. The capillary resistance is very low, usually innumerable petechiae occur at less than 10 cm. Hg negative pressure. The tourniquet test is strongly positive.

In any of these groups the chemical tests measure depletion, the capillary test, if it can be shown to respond to treatment, a morbid consequence of depletion, i.e., scurvy. In a strict sense the chemical tests cannot be used to diagnose

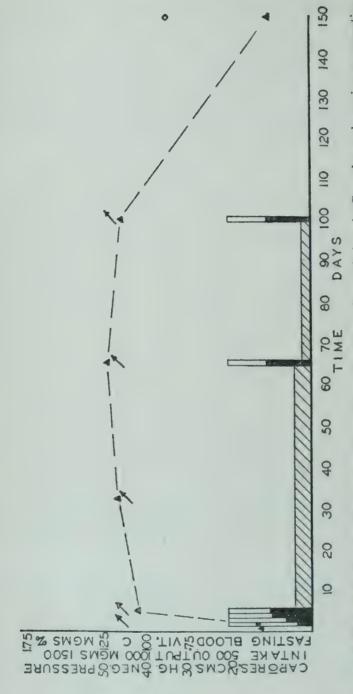


Fig. 26. The rate of saturation of an individual given large doses of vitamin C as shown by urinary excretion and rise in blood content of vitamin, the maintenance of saturation on a diet supplemented with orange juice and the subsequent depletion when the patient returned to his home.

scurvy but only to rule out that diagnosis for while scurvy has never been reported in an individual without chemical evidence of depletion ascorbic acid may be present in amounts less than 0.1 mgm. per 100 cc. without any symptoms or signs of scurvy (including capillary fragility) associated. Instances of this sort are becoming more and more numerous. Kajdi, Light and Kajdi emphasize this discrepancy and its frequency. The cases described earlier in the discussion of the incubation period of scurvy are excellent examples.

Two other procedures may be mentioned. Rotter's test in which the indicator is injected into the skin and the time required to decolorize it taken as a measure of the ascorbic acid present has been both recommended and criticized. The values are not related to blood plasma concentration. The radiographic diagnosis is often useful. Its limitation is that lesions extensive enough to be recognized radiographically occur only after depletion has been prolonged and is moderately severe. Therefore it is not as delicate as the chemical methods. Improved criteria for recognizing the radiographic lesions are discussed by Park et al.

It must be frankly admitted that no fully satisfactory method exists at present for diagnosing mild forms of vitamin C deficiency. King wrote: "As chemical methods are studied in more detail it becomes evident that each experimental method is subject to unaccountable variations from time to time. Hence, it is fortunate that a great number of methods are becoming available."

#### TREATMENT

The treatment of scurvy requires only the administration of ascorbic acid. This may be given by mouth, intravenously or intramuscularly. Several grams are required to saturate a severely depleted, scorbutic individual. In such cases the vitamin is best given in gram or half gram doses and intravenously. The dose may be repeated daily or on

alternate days. Response occurs very quickly. Goettsch has demonstrated that single, massive doses are as effective in scurvy as divided doses and that signs of repair may occur within 48 hours (calcification of subperiosteal hematomata).

For less severe deficiencies smaller doses are adequate, say

100 mgm. daily.

Much is still to be learned by the study of cases of scurvy. The apparent requirements of the particular case, the amount necessary to cure, factors which predispose to scurvy and related disturbances and lesions are all matters of lively interest. For these saturation tests, a basal diet of known ascorbic acid value and other clinical tests and measurements are valuable.

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### CHAPTER XVII

## THE NATURE AND FUNCTION OF VITAMIN D

The modern investigation of the antirachitic vitamin commenced during the war years (1914-18) and was given great impetus by Mellanby in England who studied the effect of various diets on puppies. He showed that he could produce rickets in these animals by feeding them milk and bread or oatmeal porridge. At that time an emulsion of linseed oil was widely sold in England as a cure for rickets and the casual listing of this product, which was worthless, led to the dietary study of other fats. Cod liver oil was found to be the best antirachitic of the fats tested. Since the mineral values of his stock diet were the same in all of these tests. Mellanby was led to the belief that the curative factor was not the mineral element of the diet, a favorite theory at the time, but some peculiar virtue richly present in cod liver oils. It was known that such oils were rich in vitamin A and it seemed logical to assume that this vitamin was the rickets preventive factor (Mellanby, 1921).

The study initiated by Mellanby was followed by five discoveries in particular which according to Bills were crucial in leading to our present understanding of the nature of vitamin D.

The first of these discoveries was made by McCollum and associates (1922). They proved that the antirachitic vitamin in cod liver oil was, like vitamin A, present in the non-saponifiable fraction of the oil but that it was not destroyed by bubbling air through the heated fraction while the vitamin A value was destroyed.

The second of these discoveries was that exposure of certain foodstuffs to ultra-irradiation endowed them with the antirachitic effect possessed by fish liver oils. Hess and Steenbock share the credit for this discovery.

The third discovery was that the substance accepting ultraviolet irradiation to become antirachitic substance was the sterol fraction in the oils and foodstuffs. Hess, Steenbock, and Rosenheim and Webster in England were the discoverers of this fact.

The fourth discovery was the identification of the ergosterol of Tanret (1879) as the parent substance of vitamin D. Windaus and Hess and Rosenheim and Webster are generally credited with demonstration of this substance as provitamin D, though many others participated in compiling the body of proof.

The fifth discovery was the isolation of calciferol, the active component of irradiated ergosterol in 1931 (Askew et al., Windaus et al.).

A summary of the studies leading up to the demonstration of ergosterol as provitamin D has been made by Bills and as it is concise and comprehensive we may quote it here:

Cholesterol of supposedly good purity was found to be activated by irradiation. Before irradiation, it exhibited spectral absorption in the ultraviolet region; after irradiation, it had little or no absorption. In consideration of Beer's Law, one postulated that either the cholesterol had been at least half metamorphosed, or else the substance in which the absorption spectrum was changed was a small amount of impurity which was exceedingly absorptive. It was found that repeated crystallization of cholesterol led to the accumulation of the absorbing fraction in the least soluble fraction. Furthermore, the use of very drastic means of purification, such as bromination followed by debromination, led to the production of a cholesterol which apparently had no absorption and no absorbability. The drastic means included in addition to bromine, such oxidizing agents as permanganate and decolorizing charcoals, and so it appeared that the unknown provitamin might be a highly unsaturated sterol, such as ergosterol.

Ergosterol was found to be destroyed by the same reagents which destroyed the X-substance in cholesterol. In one instance, namely in the

treatment with permanganate, even the rates of destruction of the provitamin and of ergosterol were found to be the same. Furthermore, the absorption spectrum of ergosterol was found to consist of four bands with maxima at 293.5, 282, 270, and 260  $\mu\mu$ , which are also the maxima of ordinary cholesterol. In ergosterol, the absorption was enormously more intense than in cholesterol, and the vitamin D potency after irradiation was enormously greater. Upon irradiation, the absorption bands of ergosterol underwent changes, and finally faded as did those of the impurity in cholesterol.

For some time after these observations it was believed that ergosterol was the *sole* provitamin D; that activation of foodstuffs to antirachitic potency by irradiation would be successful only if ergosterol was present; that fish liver oils owed their activity to the activated ergosterol dissolved in them.

In 1934, Waddell challenged this viewpoint and today we know that the principal provitamin D in fish oils, associated with cholesterol, is not ergosterol but another sterol likewise capable of activation by irradiation and occurring in fish oils in the activated state. We are also sure today that this sterol (7-dehydro-cholesterol) and ergosterol are not the only substances capable of acquiring antirachitic potency by irradiation. In fact, Bills (1938) claims that there are at least ten provitamins D occurring in natural sources.

Ergosterol and 7-dehydro-cholesterol are, however, the most widely distributed forms of vitamin D and when activated to antirachitic potency the vitamins so produced are today designated as vitamin  $D_2$  and  $D_3$  respectively; i.e., vitamin  $D_2$  is activated ergosterol, vitamin  $D_3$  is activated 7-dehydro-cholesterol. There is no  $D_1$  in the literature today. What was first called vitamin  $D_1$  proved to be a mixture of two sterols and the term was discarded when this was discovered. Vitamin  $D_2$  is also known as calciferol. It is generally believed today that the provitamin D present in the human skin and which is activated by exposure to the

ultraviolet of sunlight or of lamp is not ergosterol but mainly 7-dehydro-cholesterol.

#### CHARACTERISTICS OF VITAMINS D

As previously stated, of ten potential vitamins D two, activated ergosterol (calciferol or D<sub>2</sub>) and 7-dehydro-cholesterol (D<sub>3</sub>) are of prime importance in medicine today.

## Ergosterol

This substance is a characteristic sterol getting its name from Tanret's discovery of the substance in ergot in 1879. The commercially available form is today prepared from yeasts and molds in which it occurs to an amount equal to 2 per cent of the dry weight of these fungi.

It forms colorless crystals, small in size, whose melting point varies with the water content. The melting point for the nearly anhydrous form is 160°C. Commercial ergosterol usually contains about 5 per cent of an inert sterol which is not activated by ultraviolet irradiation (alpha-dihydroergosterol). Ergosterol is insoluble in water, sparingly soluble in oils, freely soluble in most of the organic solvents. On irradiation only a part of the ergosterol is transformed to calciferol (D<sub>2</sub>); ordinarily not more than 50 per cent under the best conditions.

During irradiation a series of products are formed which in order of appearance are listed by Bills as follows:

Ergosterol Lumisterol Tachysterol Calciferol Toxisterol (Substance 248) Supra-sterols I and II

Lumisterol, the two supra-sterols and calciferol have been isolated in crystalline state. Tachysterol has been separated

as a crystalline 3,5-dinitro-4 methyl-benzoate. Toxisterol has not been obtained in pure form. Early preparations of irradiated ergosterol which were irradiated too long are believed to have owed their toxic effect to production of this sterol. It is sometimes called substance 248 since it has a single absorption band at 248 m  $\mu$ .

Lumisterol according to Bills is probably not antirachitic but is converted into calciferol and it also forms with calciferol a definite addition product in the ratio of one part lumisterol to one part of calciferol. This combination was the original D<sub>1</sub> of the German investigators.

Tachysterol is also probably not antirachitic and may have a slight toxic effect.

Toxisterol has not been isolated. There is evidence that it is formed more readily when alcohol is the solvent for the solution of ergosterol to be irradiated.

The supra-sterols are not antirachitic and are only slightly toxic.

As stated above it is now possible by control of solvent and irradiation time to convert about 50 per cent of ergosterol into calciferol or active vitamin D2 without toxic products being formed. When such irradiated ergosterol is dissolved in an inert oil such as peanut or corn oil the product is known as "Viosterol." This name was coined by the Council of Pharmacy of the American Medical Association to avoid a multiplicity of trade names by individual drug houses for the same product. When this product was first marketed it was customary to express its vitamin D potency as 250 D or 100 D, etc., by which was meant that it contained 250 or 100 times the amount of D in a certain reference cod liver oil. Today vitamin D potency must be stated on labels in International or U.S.P. units, they being identical in definition. Viosterol solutions must, however, contain at least 10,000 International units of vitamin D per gram. An International unit is the vitamin D potency of 0.000025 milligrams of calciferol.

The principal commercially available forms of activated ergosterol are irradiated yeast, viosterol, and metabolized milk (milk made vitamin D potent by feeding irradiated yeast to cattle).

# 7-Dehydro-Cholesterol (D<sub>3</sub>)

For correction of rat or human rickets calciferol appears practically equivalent to the vitamin D in cod liver oil. For curing chick rickets cod liver oil has according to Massengale (1930) something like 100 times the potency of calciferol.

The provitamin D<sub>3</sub>, the unirradiated 7-dehydro-cholesterol appears to be the principal form of provitamin D in the human skin and in the active form constitutes the major vitamin in fish liver oils such as cod and halibut. It is also the principal form present in vitamin D milks produced by irradiation of the milks and in the vitamin D milks fortified by the addition of cod liver oil concentrates. It is sometimes called the normal cholesterol vitamin D since it is the form found in greatest abundance in crude cholesterol.

7-Dehydro-cholesterol was obtained in crystalline form by Schenck. Crystalline esters have also been obtained from tuna liver oil and from halibut liver oils. (Brockman, Haslewood and Drummond, Simons and Zucker.) Its absorption spectrum is similar to that of calciferol.

## Other Antirachitic Sterols

While these two forms D<sub>2</sub> and D<sub>3</sub> are the principal forms of antirachitic sterols found in our vitamin D-containing foods and medicinals there is evidence that other forms may be present. 22-dihydro-ergosterol is found in vegetable products and may be a significant factor in irradiated cereals. It has not yet been isolated from natural products. It appears to

be slightly less active for rats, weight for weight, than the other two vitamins and less active for chicks than vitamin D<sub>2</sub> (MacDonald). It was produced in crystalline form by Windaus and Trautman and has the same absorption spectrum as calciferol.

Activated 7-dehydro-sitosterol has been studied by Wunderlich and has antirachitic potency. It is present in some vegetables. Its antirachitic potency for rats is less than that of either  $D_2$  or  $D_3$ .

Cholesterilene itself cannot be activated to vitamin D potency by irradiation but cholesterilene sulfonic acid and its salts are definitely antirachitic to rats and are more potent for chicks, rat unit for rat unit than cod liver oil. The formula given is that of Stavely and Bergmann. Yoder has described the manner in which it was discovered by treating cholesterol with fuller's earth.

Of the other forms listed, little is known at present and the reader is referred to Bill's reviews (1935–1938) for their discussion.

We have no explanation as yet of how irradiation produces physiological activity. As noted in all the activated forms the phenanthrene ring appears to be broken at the position 10 and there are double bonds at this point in the ring. There is also, as we have previously cited (Milas and Anderson), evidence that the structure of the side chain enters in some way into determination of activity. How, still remains to be determined.

Milas and Anderson have reported success in the synthesis of a compound called triene which has the configuration of the active part of the vitamin D ring and study of such compounds may bring light to this problem.

### THE FUNCTIONS OF VITAMIN D

As we have noted in the discussion of the nature of vitamin D, there are now several compounds which exhibit ability to

produce antirachitic effect, the identifying characteristic of vitamin D. We also know that these compounds exhibit variation in potency with the type of animal used in testing but regardless of the test animal selected, ability to cure rickets is today our only quantitative method for determining potency. Our very definition of the vitamin D unit is based on a bioassay method using the rat as the test animal.

It follows then that a discussion of the function of vitamin D must give paramount importance to how it acts in preventing and curing rickets.

### HOW IS EXPERIMENTAL RICKETS PRODUCED?

In table 35 we have given some diets that have been used to create a condition of rickets in test animals. It will be noted that the Steenbock-Black diet 2965, the Sherman Pappenheimer diet 84, and the McCollum diet 3143, all have one characteristic in common; a relatively high calcium/phosphorus ratio. It is therefore evident that one factor in the production of this disease is the proportion of these two bone forming elements in the diet. It is also evident, since addition of vitamin D to such diets corrects the tendency to faulty bone formation, that vitamin D in some way affects utilization of calcium and phosphorus.

Shohl has reviewed the diet studies related to rickets production. Historically he shows that McCollum and his collaborators and also Sherman and Pappenheimer were in agreement that when the ratio of calcium to phosphorus in the diet was of the order of 4/1 or greater rickets developed and McCollum also showed that rickets was producible by the reverse condition, viz. low calcium/high phosphorus ratio in diet. When the proportions were nearly equal no rickets developed.

From these observations it came to be held that a faulty calcium/phosphorus ratio was the primary cause of rickets. But Shohl and his collaborators showed that the absolute

amounts of calcium and phosphorus eaten as well as the ratio between them was a factor in rickets. As absolute amounts of calcium and phosphorus are diminished the degree of rickets becomes more intense; not only for the high calcium, low phosphorus diets and the low calcium, high phosphorus diets, but also for low calcium, low phosphorus diets in spite of ratios of 2/1 or 1/2.

TABLE 35
Rickets Producing Diets

| Sherman-Pappenheimer's Diet #84 Patent flour Calcium lactate. Na chloride Iron citrate.                         | 95%<br>2.90<br>2.00<br>0.10          | Ca/P ratio: 6.5/1 |
|---|--------------------------------------|-------------------|
| Steenbock-Black Diet #2965 Yellow corn Wheat gluten Calcium carbonate Sodium ehloride                           | 76%   20%   5%   1%                  | Ca/P ratio: 4/1   |
| McCollum Diet #3143 Whole wheat Whole yellow corn Wheat gluten Gelatin powder Calcium carbonate Sodium chloride | 70%<br>33%<br>15%<br>15%<br>3%<br>1% | Ca/P ratio: 4/1   |

Shohl also points out that since both calcium and phosphorus are necessary for bone formation any factor which influences the supply or utilization of these elements can be an influence in bone formation. On that basis he would include as rachitogenic factors metals forming insoluble phosphates (beryllium, magnesium, strontium, iron, lead, thallium). We also know that among such factors we must include the acid/base ratio of the diet for with a diet in which the acidic radicals predominate the calcium salts are made more soluble

and are hence more effectively absorbed. Nicolaysen claims that vitamin D increases calcium absorption from the gut and not the absorption of phosphorus.

Shohl also calls attention to the work of Hamilton and Schwartz in converting rachitogenic diets into normal diets by the addition of organic acids and alkaline ash and the production of rachitogenic diets by adding alkalies plus acid ash to normal diets. In the first mentioned diets, Hamilton and Schwartz used sodium acetate, sodium tartrate, sodium bitartrate, citric acid, and tartaric acid in the order of their effectiveness. In the second series they used ammonium carbonate and ammonium chloride. Shohl holds that their effects with the organic acids were not simply due to the acidity produced but that the nature of the organic acid ion played a rôle and that the citrate ion is definitely more pronounced in effect than the tartrate ion. Citric acid plus alkaline residue added to rachitogenic diets prevented and cured rickets.

It is evident therefore that there are several ways to control the behavior of ingested calcium and phosphorus other than by ratio control and that in looking for the action of vitamin D on this control we must consider a number of ways in which it might act. Furthermore, since calcium and phosphorus can reach the bone only by way of the blood supply we must investigate its action on blood.

It is admitted that swallowed calcium and phosphorus after absorption from the intestines must follow one of three routes: it may be deposited in the tissues; it may be excreted into the gut and ejected with the feces; it may be excreted in the urine. In clinical rickets there is increased excretion of fecal calcium and decreased urinary excretion. Fecal phosphorus excretion is also increased.

## WHERE DOES VITAMIN D ACT?

One of the earliest diagnostic signs of rickets to be used to determine the effect of a potential source of vitamin D was a

measure of the inorganic phosphate content of the blood serum (Hess, 1933). This value lowers appreciably in rickets and is restored to normal by vitamin D administration. Calcium content of blood is also lowered in rickets and raised by vitamin D but not to the extent of the variations in inorganic phosphate (see table 36).

Measurement of blood phosphate is not today considered very satisfactory as an index of the beginning of healing since healing often starts (shown by roentgenograms) some time before restoration of normal blood phosphate content. Such tests would suggest that vitamin D acts primarily in controlling the blood content of phosphorus.

TABLE 36
Calcium and Phosphorus Content of Blood

| CLINICAL CONDITION | P PER 100 CC. | Ca PER 100 cc. |
|--------------------|---------------|----------------|
|                    | mgm.          | mgm.           |
| Rachitic, no D.    | 3             | 7              |
| Normal, with D     | 4.5           | 10             |
| Hypervitaminosis D | 8             | 15             |

Robison first reported the discovery of an enzyme called phosphatase. This enzyme was shown to have the power to split off inorganic phosphate from organic phosphoric acid esters such as hexose phosphates and glycero-phosphates. It was later shown by Kay and others, that in bone diseases of various types, including rickets, there is an accumulation of this enzyme in the blood (see table 37).

Its presence in the blood is not apparently associated with any function it performs in that fluid and hence Kay considers that it is there because of "leakage" from tissues of higher content.

It is obvious that in the bone tissue this enzyme would be useful in converting organic phosphates into the inorganic phosphate ion necessary to precipitation of calcium phosphate and hence its presence in the blood would mean that it was draining away from where it was needed. Also since addition of vitamin D restores blood phosphatase to normal perhaps the

TABLE 37
Blood Phosphatase in Bone Diseases
(After Kay, 1932)

| CONDITION                               | NUMBER OF<br>CASES | AVERAGE PHOSPHATASE<br>CONTENT OF THE<br>PLASMA |
|---|--------------------|---|
|   |                    | units   |
| Normals                                 | 33                 | 0.14  |
| Arthritis with bony changes             | 7                  | 0.17  |
| Osteomyelitis                           | 8                  | 0.27  |
| Myositis ossificans                     | 3                  | 0.17  |
| Fragilitas osseum (Infants or children) | 6                  | 0.41  |
| Rickets infantile                       | 13                 | 1.03  |
| Rickets renal                           | 2                  | 1.20  |
| Rickets adolescent                      | 1                  | 2.4 or more                                     |
| Osteitis fibrosa generalized            | . 3                | 1.8   |
| Osteitis fibrosa focal                  | 7                  | 0.21  |
| Osteitis deformans                      | 24                 | 1.7   |

TABLE 38

Influence of Vitamin D from Several Sources on the Serum Phosphatase of Chicks (After Correll and Wise, 1938)

Groups of chicks: #23

| PHOSPHATASE PER 100 CC. OF SERUM | VITAMIN D PER 100 GRAMS OF DIET |                             |                             |                              |
|----------------------------------|---------------------------------|-----------------------------|-----------------------------|------------------------------|
|                                  | None                            | 18 I.U. as cod<br>liver oil | 37 I.U. as cod<br>liver oil | 37 I.U. as tuna<br>liver oil |
| On 1st day                       | 71.3                            | 71.3                        | 71.3                        | 71.3                         |
| In 2 weeks                       | 158.7                           | 56.4                        | 69.6                        | 81.3                         |
| In 4 weeks                       | 267.7                           | 44.1                        | 41.4                        | 65.0                         |
| In 6 weeks                       | 248.0                           | 54.8                        | 48.2                        | 115.2                        |
| In 8 weeks                       | 240.0                           | 44.0                        | 38.6                        | 76.6                         |

vitamin controls the behavior of this enzyme. Correll and Wise have produced evidence to show the marked effect of vitamin D administration in chicks on blood phosphatase content (see table 38).

The discovery of the effect of vitamin D on blood phosphatase content led to the hope that it might prove a more acceptable diagnostic test for healing of rickets than the blood phosphate test but today, while increase in phosphatase appears to develop before increase of phosphate and thus makes the test an earlier indication of rickets than the phosphate test or the roentgenogram, it does not give satisfactory indication of the starting of healing for that takes place in many cases several weeks before the blood phosphatase begins to decrease significantly. Looney has reviewed the present status of this test and its relation to rickets.

#### VITAMIN D AND CELL RESPIRATION

Rachitic animals show a lowered basal metabolism when compared with normal animals and when such rachitic animals are cured by administration of vitamin D basal metabolism is restored to normal at the same time. This would indicate that in some manner vitamin D plays a part in the intercellular oxidation system, in some way acts directly on tissue cells. The work of Presnall has shown that skin sections taken from rachitic rats and from normal rats and tested by the Warburg oxygen absorption method show a contrast in the ability to take up oxygen. The skin of rachitics has a lower oxygen uptake. Here again there is a suggestion that D acts in the tissue cell itself.

Reed has suggested that it may be that this effect is accomplished indirectly by vitamin D acting on the anterior pituitary gland and causing increased secretion of the thyreotropic hormone.

We have then evidence of vitamin D exerting an influence on the inorganic phosphate content of the blood. We have evidence that it is related to the distribution of phosphatase and we have evidence of its relation to tissue respiration but we have as yet no completely satisfactory explanation of where vitamin D actually functions.

Harris and associates claim that vitamin D increased net

absorption of calcium and phosphorus from the gut. cently there was developed a new method of following the fate of phosphorus in the body. This was accomplished by using as a part of the food phosphorus, a special isotope P<sub>32</sub>, which is radio-active. By feeding this and examining with x-ray it is possible to follow its progress through the body and its deposit in specific regions of the body. Morgareidge and Manley used this isotope and claim that the addition of vitamin D did not increase the absorption of phosphorus. Feeding the P<sub>32</sub> with sodium phosphate, they showed that the amount appearing in the blood was the same for rachitic and vitamin D treated rats but while the amount of the phosphorus appearing in the bone was the same for rachitic and vitamin D rats for the first 54 hours, after giving the dose the amount in the metaphysis of the D rats rose 2 per cent higher while in the rachitic rats the rise was only  $\frac{1}{2}$  per cent. These tests would indicate vitamin D actually controls delivery of phosphorus to tissue where it is needed but not by increasing the rate of its absorption from the digestive tract.

In Chapter III we called attention to the fact that utilization of fuel in tissues involves not only presence of oxidizing enzyme systems but of phosphorylating systems. In other words, before sugar can be oxidized not only must the hydrogen be activated to leave the sugar and the oxygen activated to receive it but this does not take place unless the sugar is first phosphorylated. Phosphorylation is accomplished by phosphatase enzyme systems. It would seem possible that vitamin D might form a part of such a phosphorylating enzyme system just as vitamins B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, etc. have been shown to be parts of a hydrogen acceptor system. This point would seem to be worthwhile investigating further.

# WHAT IS ESSENTIAL TO NORMAL BONE FORMATION?

Bone is a mineral substance occurring in nature in a form form called dahlite. The chemical formula of dahlite is nCa<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>: CaX in which X may be CO<sub>3</sub>, OH, or F and n

may be 2 or 3. In bone X is usually  $CO_3$  and the ratio of  $Ca_3(PO_4)_2$  to  $CaCO_3$  is usually two mols of the former to one of the latter. By analysis bone salt is 12 per cent calcium carbonate and 88 per cent calcium phosphate. For bone salt to precipitate out from a solution there is necessary not only a certain concentration of these two salts but also a certain ratio between them. Howland and Kramer suggested using the production of the blood calcium and phosphorus values as an index of presence or absence of rickets. By table 36, the normal concentrations are 10.5 Ca to 4.5 phosphorus or a product of approximately 50 (10.5  $\times$  4.5 = 47.25). They reported that rickets occurred when that product was less than 40 and that healing commenced when the product reached 40.

McLean and Hastings state that if the ion product of Ca  $\times$  PO<sub>4</sub> is less than  $10^{-27}$ , Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> will go into solution; that to initiate precipitation the product must not exceed  $10^{-23.5}$  but that once started precipitation will continue until an ion product of  $10^{-27}$  is reached. This, provided the proportion of solids to fluid in the solution is greater than 150 mgm. per liter. By this solubility theory of bone formation the problem is one of securing a precipitable condition for the Ca and P ions. If vitamin D controls the concentration of phosphatase in the bone it would then function by the following steps; proper phosphatase concentration plus a supply of organic phosphate would enable the enzyme to split off enough phosphate ion to secure the precipitation product between Ca and P and the deposition of the bone salt.

We can see from this discussion that control of bone salt ions precipitation is somehow accomplished by vitamin D administration even when the proportions of calcium and phosphorus in the diet are unfavorable to this precipitation but how a sterol such as vitamin D actually accomplishes this action is still unexplained.

# OTHER FUNCTIONS OF VITAMIN D

Calcium and phosphorus serve other purposes in the body than the formation of bone. If vitamin D controls the blood content of calcium and phosphorus it obviously is a factor in controlling behavior that is influenced by these elements. Some of this behavior is susceptible to calcium and phosphorus supply throughout life, hence there is excellent reason for suggesting that vitamin D is required by the human being throughout life. We know definitely that osteomalacia (morbid softening of the bone) and the hunger osteopathy of adults can be prevented and corrected by vitamin D therapy. Pregnant women need additional supplies to prevent loss of calcium from the tissues and teeth.

There is cumulative evidence (MacBeath) to show that vitamin D is at least one factor in the prevention of dental caries though not the only one.

There is also evidence that vitamin D is a factor in the control of tissue cell respiration. It has been known for some time that rachitic animals show a lowered basal metabolism. Presnall has shown that topically applied vitamin D has the power to increase the oxygen uptake of the skin of rachitic rats and this ability has been confirmed by Göthlin. Certain skin diseases have been shown to benefit by use of vitamin D therapy, for example, acne (Doktorsky and Platt) and psoriasis (Ceder and Zon) and perhaps it is by influence on cell metabolism that the D is effective. We were able to demonstrate by growing chick epithelial cells in vitro that addition of vitamin D to the culture medium in the presence of adequate vitamin A would definitely stimulate the growth of the cell mass. D appears then to be a significant factor in cell metabolism, especially in skin metabolism.

In celiac disease much undigested fat (steatorrhea) and calcium salts are lost in the feces. Vitamin D by mouth or ultraviolet irradiation has been reported beneficial in such cases.

Keratoconus is the name given to a hyperbolic bulging of the central part of the whole of the cornea, accompanied with inflammation. Knapp reports that this condition is producible in dogs by feeding them a diet deficient in vitamin D and calcium content and that cases of this disease respond to vitamin D therapy.

These clinical aspects of vitamin D function are dealt with

more extensively in the following chapters.

#### HYPERVITAMINOSIS D

In large amounts vitamin D can produce toxic effects. According to Park, however, "The rule holds that the dose of vitamin D will not become toxic so long as the calcium and inorganic phosphorus levels in the blood are not affected. Apparently the toxic action does not depend upon the level of vitamin D in the blood but rather on its effects on the calcium and phosphorus metabolism." Park further states that:

The physician should be on his guard for signs of toxicity in the child if the daily dose exceeds 50,000 units. If the patient is a very small infant, e.g., a premature infant, the physician should watch with care even if the dose is 20,000 or 30,000 units. Toxic manifestations will not occur immediately but only after a period of two or three weeks and need not be expected so long as the rickets, if this condition is present, remains unaffected. With the adult the physician ought to exercise great caution in venturing into the unknown with daily doses of 200,000 units or more.

The toxic dose then is of a very considerable magnitude and far in excess of what ordinary vitamin D intake is liable to be from use of vitamin D foods or lamp irradiation. A quart of fortified vitamin D milk ranges from 135 units per quart for the irradiated type to 400 to 450 units per quart for the metabolized and fortified milks. U.S.P. cod liver oil contains only 85 units per gram and three teaspoonfuls would give one only 12 times this amount or 1020 units. It is therefore only in large doses that toxic effects need be feared and such dosage

should of course not be given except under the attention of a qualified physician. Jeans puts our average daily need throughout life at probably not more than 400 units per day.

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## CHAPTER XVIII

# VITAMIN D DEFICIENCY

Vitamin D deficiency most conspicuously affects mineral metabolism and most characteristically and importantly results in rickets. But in two respects this avitaminosis differs from others. In the first place dietary intake is supplemented and may be largely replaced by endogenous synthesis. In the second place the effect of the deficiency is intensified or minimized by mineral supply and this is dependent quite as much on intake, requirements and the function of other organs as on vitamin D. Therefore the vitamin is not always of decisive importance.

The synthesis of vitamin D in the body occurs in the superficial layer of the skin through the action of certain solar rays. This has been demonstrated by the irradiation of excised portions of skin (Luce-Clausen). The first effect of its formation, or its ingestion, is an increase in blood phosphorous which is followed by lime salt deposition in the bones. Vitamin D presumably has nothing directly to do with the latter but functions only to increase calcium absorption from the intestine or to increase phosphorous retention which in turn increases calcium retention. Naturally calcium and phosphorous must be present in adequate amounts as well as certain organic acids (Shohl).

In practice, however, the mineral intake is much less important than the supply of vitamin D and the effect of the latter is so great that relatively unfavorable mineral conditions may be corrected by adequate amounts of vitamin D. Nevertheless experimental rickets is induced not by vitamin

deficiency alone but by vitamin deficiency plus a relative deficiency of calcium or phosphorous or an absolute deficiency of either or both. Spontaneous rickets is usually a low phosphorous rickets.

We have mentioned that vitamin D is effective only through the mechanisms of mineral metabolism and therefore dependent upon them. This is best demonstrated by reference to rickets caused by feeding cations which by forming insoluble phosphates intercept calcium in its progress to the bones. Beryllium will do this as Branion, Guyatt and Kay have shown in rats. It is the probable explanation of Caffey's case in which an infant receiving adequate amounts of cod liver oil but suffering from lead poisoning developed rickets. An interesting relationship between the metabolism of lead and calcium is seen in cases of this kind since the usual lead deposit in the bones is missing just as calcium is.

Nephroselerosis is associated with an uncommon condition in infancy in which a mineral disturbance and lesions resembling those of rickets occur. The theory has been proposed that phosphorous is retained in these cases and that low blood calcium is due to high blood phosphorous (which in contrast to dietary rickets is regularly present). Mitchell, however, suggested that phosphorous retention increases the intestinal excretion of phosphates and that these precipitate intestinal calcium and prevent its absorption. Pappenheimer demonstrated that a low calcium diet fed young rats in which kidney tissue had been reduced in amount produces a stunted growth and skeletal lesions resembling "renal rickets." Many cases of "renal rickets" are due to hyperparathyroidism.

Mention must also be made of the views of Mellanby who has emphasized the significance of certain cereals in the production of rickets. Mellanby's views arose from the observation that cereals varied greatly in their ricket-producing qualities and that those with higher phosphorous and calcium

content, such as oatmeal and wheat germ, were more liable to produce rickets than rice and white flour. Mellanby has ascribed these reactions to the presence of an "anti-calcifying toxamin." A more popular opinion is that experimental diets predominantly containing such cereals induce rickets because they contain phosphates in the form of phytin which is poorly absorbed. This in no way detracts from the significance of Mellanby's views to the present discussion and serves equally well to demonstrate how other factors, of which perhaps but a few are yet known, determine vitamin requirements and whether lesions ordinarily ascribed to vitamin deficiency do or do not develop despite "adequate" intake.

In 1933 Gerstenberger reported cases of rickets in infants suffering from biliary obstruction (congenital obliteration of the ducts). These were refractory to cod liver oil treatment. The circumstances have been reproduced in rats by Heymann both by ligation of the bile ducts and liver necrosis from carbon tetrochloride poisoning. Under such circumstances the requirement of vitamin D, as judged by the cure of rickets, is increased from 10 to 12 times the normal amount. Whether this is wholly due to faulty absorption through lack of bile or whether the liver operates in some other manner is not known.

Other forms of rickets have been described which are refractory to vitamin D. These have lately been cited by Park. In certain cases the refractory state persists for long periods. Albright, Butler and Bloomberg have reported the natural history of such cases including one observed for 14 years. The failure to respond appeared due to an intrinsic resistance to vitamin D since the refractory state remained after absorption had been circumvented by intravenous medication and ultraviolet radiation. In some of these cases at least the resistance to vitamin D effect is only relative, as is true of so many related deficiency diseases, and can be overcome by large doses.

# THE MORBID ANATOMY OF VITAMIN D DEFICIENCY

Pathologic anatomy has been of inestimable value in studies of vitamin D deficiency. The anatomical criteria are easily recognized and have served to guide many valuable experimental studies. Rickets was well understood, from an anatomic standpoint, long before the period of vitamin research and the older knowledge of rickets had only to be applied to the induced forms of the disease in dogs and rats to afford accurate means of measuring the effects of various diets and antirachitic agents.

The anatomic difficulty today is largely dependent on the fact that similar lesions occur in other periods of life than infancy and under various conditions. In such cases they are frequently too imperfectly formed to permit precise diagnosis. The problem today is one of determining the rôle of vitamin D in such borderline states and the anatomic approach to this problem has yielded relatively little new information.

At least three diseases share the pathologic anatomy characteristic of deficiency of vitamin D. They are rickets, by far the most common of the conditions, adolescent rickets and osteomalacia. Pathogenetically, histogenetically and etiologically no fundamental differences exist between them. Combination forms are not uncommonly seen in animals. In such cases the bones which have ceased to grow, for example the bones of the extremities, develop osteomalacic lesions while the still growing ribs become rachitic. Late, or adolescent rickets frequently constitutes a similar transitional state in man.

The gross differences between rickets and osteomalacia are due to the presence or absence of growth in the affected bones. Growth, furthermore, determines the degree of the rachitic lesions, the two being strictly proportional. The most apparent rachitic lesions, at sites of enchondral bone growth, are lacking in osteomalacia simply because enchondral bone growth is lacking. The relative paucity of osteoblasts in osteomalacic

lesions is due to the normally fewer osteoblasts in the bones of adult animals. The tendency for fractures to occur in osteomalacia while bending is the rule in rickets is due to the persistance of some rigid, calcified bone in the former, a survival from healthier days and the natural difference in rigidity between the bones of infants and adults.

Rickets and osteomalacia share another significant feature. Both are closely related to normal physiological tides. Rickets is an exaggeration of changes which usually occur in the winter months and represents an imbalance of forces constantly at work in growing bones. Schultz, for example, has insisted that the experimental criteria of a normal costo-chondral junction are false and represent a stage of over-dosage of vitamin D. In the case of osteomalacia the process seems pathological only in the degree to which it exceeds the normal changes in pregnancy. Whether normal in either case is identical with optimal conditions is, of course, open to serious doubt but the point remains that both processes represent exaggeration of disturbances of bone growth which are the rule among our people.

The confusion which still exists concerning the pathogenesis of osteomalacia seems to us to be due to a number of causes. In the first place the disease is relatively infrequent and has not been studied as thoroughly as rickets. In the second place precise diagnostic methods have seldom been applied to cases of osteomalacia. The clinical attitude has been that unquestionable cases are those instances of softening of the bones during adult life without definite accompanying bone disease. By these standards other conditions and especially osteitis fibrosa must frequently have been confused with osteomalacia. The third cause for confusion has been the successful treatment of many cases by castration, adrenalin and pituitary extracts. The former is said to be effective in almost all cases and reversal of the negative calcium balance has been observed following bilateral oophorectomy.

Too many glands besides the parathyroids have been implicated to maintain the theory that these cases are due to hyperparathyroidism. Moreover the chemical studies are in complete accord with the known facts concerning rickets, reduced plasma phosphorous, increased phosphatase etc. We believe therefore that osteomalacia is essentially the adult counterpart of rickets but that other osteo-dystrophies have frequently been confused with it and that, in the past, a variety of conditions have masqueraded under the title of osteomalacia. The problems associated with the pathogenesis of osteomalacia will probably not be fully solved until the association of parathyroid hyperactivity and rickets is solved.

Blumgart, Gargill and Gilligan came to the same conclusion after intensive metabolic study of a case of osteomalacia. The mineral balance, the retention of calcium and phosphorous when vitamin D or ultra-violet light was given was similar to that occurring in rickets. The hematologic and radiographic features of their case were also closely related and they concluded their study with the comment that "osteomalacia, as manifested by this patient, is a form of adult rickets."

In a recent essay Mellanby reports the production of osteomalacia in dogs by dietary means. The photographs of the ribs are very convincing but other data are not given.

Of the other conditions which share the histologic lesions of rickets the most interesting is deficiency osteoporosis or "hunger osteopathy," a disease which became conspicuous in Vienna during the World War. The similarity of this condition to osteomalacia emphasizes the unsatisfactory terminology employed for these bone diseases all of which seem to be essentially the same.

In deficiency osteoporosis the bone pains of osteomalacia are prominent and are associated with similar, if less well marked, changes in the long bones. Bending and fracture of bones may occur. The cases reported seem to have been the late effects of sharply restricted diets. They respond favorably to cod liver oil. It would seem that these cases might be considered as transitional forms between osetomalacia and senile osteo-porosis.

The lesions in spontaneous rickets in man and other mammals are frequently very complicated due to secondary factors such as stresses and strains and to variable dietary and hygienic effects. Such lesions are naturally less suitable than experimental lesions for purposes of demonstration. For a discussion of the lesions of human rickets Pommer's monograph remains a classic contribution and the recent volume by Marek and Wellman expands the field by including studies of the lesions in a variety of domestic animals as well as man. The purposes of this book will be served by a description of the morbid changes in experimental rickets. A description of the gross lesions in rickets and osteomalacia is included in the discussion of the clinical features of those diseases.

## THE PATHOLOGICAL ANATOMY OF EXPERIMENTAL RICKETS

The histologic details of rickets have been studied to best advantage in experimental animals where the duration and the degree of the deficiency may be controlled. Judged by such material the fundamental disturbances seem to consist of abnormal cartilage involution and faulty calcification of osteoid matrix. These changes occur only in growing bones for rickets is a disturbance of growth. It is interesting however to note that histologic study quite clearly shows that neither the osteoblasts nor cartilage cells are retarded in their rate of growth or proliferation. It is rather that their functions are disturbed.

Bone growth consists of proliferation of cartilage cells on their epiphyseal side and degeneration on the diaphyseal side. The constantly advancing line of cartilage cell degeneration is invaded by capillaries and osteoblasts which deposit osteoid tissue on the remnants of the cartilage matrix. This form of bone growth, enchondral bone formation, is characteristic of skeletal development for it produces elongation of the bones by constantly organizing osteoid tissue behind the retreating line of cartilage.

The first feature of skeletal rickets is observed in the progressively involuting cartilage cells, which, as they degenerate in advance of osteoid organization, become enlarged, clear and empty looking. Wolbach has studied these changes in great detail and describes the earliest lesions in rickets as follows:

"The first histologic evidence of rickets is the absence in whole or in part of the layer of clear cells and the consequent absence of ingrowth of capillaries. Slight degrees of rickets are manifested by a moderate increase in width of the epiphyseal cartilage presenting an irregular border on the diaphysial side. This irregularity is due to the fact that the cessation of degenerative sequence in the cartilage cells does not take place simultaneously over the diaphysial border. The width of the epiphyseal cartilage continues to increase because of the continued activity of the proliferative zone and the survival of the cells on the diaphysial side.

"In rickets, after the cessation of the degenerative sequences of the cartilage cells, calcification of the cartilage matrix ceases, and osteoid material accumulates around the capillaries of the diaphysis adjacent to the cartilage. Osteoid material increases in amount with the duration of the dietary deficiency and, being non-calcified, is molded by the pressure of weight bearing."

The redundant cartilage and excessive osteoid result in an enlargement of the zone of growth and in weakness. The lesion is highly vascularized and pinkish in color. Since the uncalcified osteoid also forms beneath the periosteum and within the bone the entire bone, to a lesser extent, enlarges and is weak and soft in contrast to the scorbutic bone which is fragile and thin. Dodds and Cameron have described the metaphysis in rickets in detail. They consider the lesions to be characteristic and important. The tissues corresponding to the primary spongiosa of normal bones are formed of cartilage remnants on which are deposited considerable amounts of osteoid. These masses of osteoid may be identified by the uncalcified cartilage matrix within throughout the healing process.

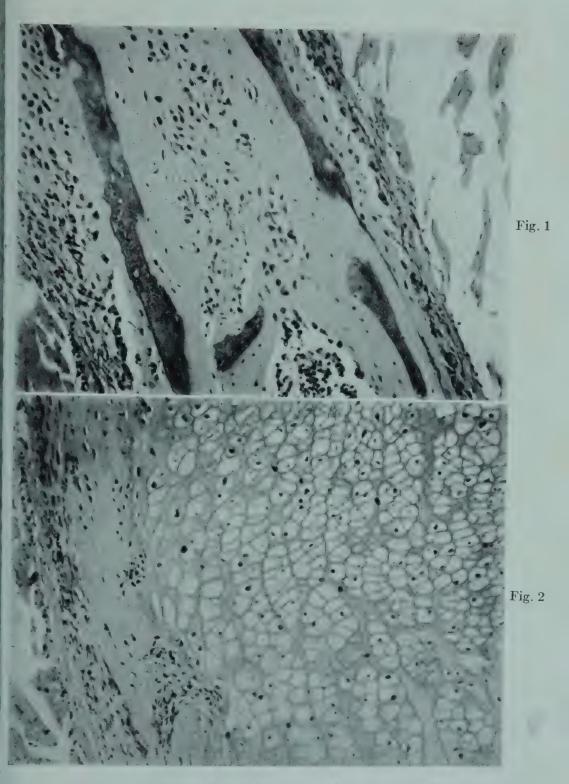


PLATE XXXVI. Rachitis. Figure 1 from the rib of a rachitic rat. The great quantity of homogeneous, uncalcified osteoid tissue is seen in prolonged rickets. Figure 2 is from a similar animal and shows the appearance of the redundant cartilage masses which form in vitamin D deficiency.



PLATE XXXVII. Experimental rickets. Three stages in the development of a rachitic lesion at a costo-chondral junction. Photographs made at same magnification from animals of same size and on same diet but examined at increasing periods. Notice the overgrowth of cartilage and osteoid tissue within the bone and about the periosteum. Notice the increasing size of the junction which is responsible for the rachitic "rosary."

The first signs of healing are the deposition of calcium in the cartilage, usually seen within twenty-four hours after giving cod liver oil (Pappenheimer) and the reappearance of clear, degenerative cartilage cells on the diaphyseal side of the cartilage plate. Wolbach states that this effect is also noticeable within twenty-four hours. Lime salts are also promptly deposited in the osteoid material. The capillaries commence to invade the degenerate cartilage (within forty-eight hours, Wolbach) and the formation of natural bone trabeculae occurs.

Calcification, in recovery, does not develop uniformly but in scattered foci. Hess stated that the earliest calcification appeared not at the metaphyseal margin of the redundant cartilage but within it, where the normal zone of calcification should be. Dodds and Cameron disagree with this. They found the earliest calcification in the metaphysis and that it reached the level designated by Hess only after healing was well advanced. They also stated that a characteristic phenomenon of healing rickets was that calcification first spreads towards the shaft in a reversal of the normal direction. Only when healing is far advanced is the direction restored to normal.

An early sign of healing is the invasion of the degenerating cartilage by capillaries which occurs within 48 hours of treatment. This erosion commences from the lateral margins. The deposition of lime salts is the basis of the commonly used "line test" in which the bone is soaked in silver nitrate solution and then exposed to light. The calcium deposits so revealed are routinely used in gauging the presence and extent of healing.

Rachitic bones appear to be enlarged near the joints but this may be only a relative enlargement due to cessation of growth of the adjoining cortex. Dodds and Cameron support this view with measurements of rachitic and normal rat bones. The chemical composition of the rachitic bone is similar to that in scurvy. The amount of ash is less but the calcium phosphorous ratio is normal indicating that if these minerals

are deposited at all they are deposited in the usual form. Growth of long bones is retarded very early in rickets but continues at a slower rate for three weeks when it ceases alto-

gether (Dodds and Cameron).

Histologic aids in the diagnosis of rickets include the presence of excess osteoid tissues, best recognized in the shaft. "Presence of osteoid in excess is a cardinal sign of rickets and is pathognomonic of the disease. It is the only sign in osteomalacia and may be the only sign in the older child" (Park). But rickets may be present without excess of osteoid to indicate its presence. Failure of lime salt deposition is an early sign. This may be only focal, consisting of gaps in the normal line of calcification. Distorted or crushed cartilage cells and excess cartilage cells should be searched for. The general structure of the metaphysis is that of great disorder due to irregularities of growth and the complications caused by stresses in an insecure organization.

The teeth in experimental rickets reveal the same lack of calcification seen in the bones. In this case matrix continues to form, but is not naturally calcified. One of the earliest changes is that the globules of lime are reduced in size, and uncalcified spaces appear in the dentine. Except in severe cases, the odontoblasts are not affected as far as can be judged from their appearance. The earliest changes in the teeth of rats occur within the first week according to Becks and Ryder, and consist of a distinct widening of the predentinal layer. The layer is irregularly filled with globular masses of lime salts. Subsequently, calcification ceases entirely, and the matrix occasionally is seen invaded by small vessels, a feature which is uniformly present after prolonged rickets. Becks and Ryder consider that the primary effect of the deficiency is on the odontoblasts, not due to lack of lime salts, but rather to lack of vitamin D, and by analogy they feel that the same is true of the lesions in the bone.

In Mellanby's original observations of experimental rickets

hypertrophy of the thyroid gland was described. A wide variety of studies have since been made of the condition. Thompson has said that the hypertrophy of the thyroid gland in rickets was due to dietary deficiency in iodine and could be prevented by feeding small amounts of iodine. This hardly explains other results. For example Bergman showed that active secretion developed in rats raised in darkness and that the secretory phase changed to one of colloid storage when they were exposed to light. Nitschke demonstrated a similar effect of viosterol on the thyroid. These must have been due to vitamin D.

Nitschke and Doering explored other phases of the problem. They found infant rickets regularly accompanied by low blood iodine concentrations which returned to normal after 10 to 20 days of treatment. In florid rickets the concentration of iodine in the blood fluctuated between 2–5  $\gamma$  per cent, lower values than occur in myxoedema. In normal children the value was between 7.5–13.5  $\gamma$  per cent. These authors also state that thyroxin intoxication is associated with an increase in blood phosphates indicating that a close relationship exists between phosphates and thyroid function.

The parathyroid glands are enlarged and hyperplastic in experimental rickets, in rachitic infants, and in osteomalacia. Erdheim's original observations have been repeatedly confirmed. Various explanations for this association of parathyroid hypertrophy and rickets have been suggested. The concensus seems to be that the glandular enlargement is compensatory and plays no significant rôle in the rachitic process. Ham and Lewis have recently explained related experimental results by considering the action of vitamin D as intermediary in the parathyroid mechanism. The point is unsettled. Several significant reports may serve to indicate the complexity of the problem.

Chicks deprived of vitamin D to a degree insufficient to produce rickets develop parathyroid hyperplasia. On the other hand a partial deficiency of parathyroid hormone intensifies a deficiency of vitamin D and increased parathyroid hormone reduces the requirements of the vitamin. But of greater significance than these interesting quantitative studies is the demonstration of Pappenheimer that vitamin D is antirachitic in parathyroidectomized rats. The rôle of these incretory glands is evidently of secondary importance.

The most recent contribution to the subject are the rat experiments of Ham et al. which seem to show that parathyroid hypertrophy is not a feature of rickets per se but only of low calcium rickets. In their animals low phosphorous rickets was not associated with hypertrophy. Moreover hypertrophy was produced by diets which caused the blood calcium levels to remain low, in the absence of rickets, but not by diets which caused high blood phosphorous values.

#### EXPERIMENTAL RICKETS AND CONSTITUTIONAL FACTORS

The lesions in experimental rickets are less constant than those in other deficiency diseases. The variable susceptibility of individual animals has been credited to constitutional differences. In some cases the maternal diet has been responsible. In Grant and Goettsch's investigation of this problem it was discovered that female rats could be depleted of their vitamin stores and that once this had occurred their litters developed rickets more rapidly and to a greater degree than normal animals. Similar results have been reported by the Toveruds in dogs. That this mechanism, of maternal nutrition, does not explain all of the cases of susceptibility or resistance to rickets has been shown by Hess and Blackberg who fed identical diets to four puppies from one litter. Two of the mongrels were short haired and two long haired and other constitutional differences were evident between the two pairs. The two short haired terrier dogs developed more severe rickets than their litter mates. Stockard has observed constitutional differences among dogs and a pronounced difference

between sexes. According to Stockard it is difficult to raise male St. Bernards with normal skeletons while the females seldom develop rickets.

### THE MORBID ANATOMY OF HUMAN RICKETS

The rachitic deformities depend on the mechanical influence of the weight of the body on the weakened bones. The diaphyses bend outward in active children, the femure also arching forward as well as laterally. Extreme bending can also occur near the joints and result in such deformities as genu valgus. Angulation in the soft osteoid tissue leads to common chest deformities in which the costo-chondral junctions are sharply depressed and the sternum pushed ventrally to form a "pigeon breast."

The skeletal lesions vary in their gross appearance with the extent of the disease and its distribution. If the periosteal and endosteal osteoid tissue is extensive the bone cortex is soft, pinkish in color and easily cut. In other cases the cortical changes are absent or insignificant. The epiphyseal lesions, once they are transected, show the characteristic chondral overgrowth and the heavy osteoid masses which fill the end of the marrow cavities. The skull may show osteoid masses growing beneath the periosteum as well as large fontanels and defects in the skull bones.

The histologic changes need not be given. They would only recapitulate what has been said of the bony lesions in rat rickets.

The lesions in human teeth are seen in the permanent dentition since rickets occurs during the calcification of these teeth. Dimpling of the enamel, furrows, wave-like and point defects occur (Freudenberg). The available evidence all points to the important rôle of vitamin D in the development of the teeth (Schultz) though it should be remembered that the effects of vitamin A deficiency on the enamel organ have been relatively neglected in the studies of human dentition and

some effects ascribed to vitamin D may have been due to vitamin A as well.

Be that as it may the association of rickets, formerly so common, with equally common developmental defects in teeth which were being formed at the precise time when rickets is most noticeable is highly significant.

Hypoplastic defects of the milk teeth are also very common. May Mellanby found only 21 per cent of 1260 deciduous teeth to be normal in structure. The dental defects described by Mellanby are similar but less extensive than those since found in established cases of foetal rickets. Wolfe's report may be consulted for illustrations of deciduous teeth stigmatized by rickets occurring before birth. The location of the dentin and enamel defects could be correlated with the period at which these parts are known to form and from such information the probable period of intra-uterine life at which the rickets occurred could be estimated.

Enlargement of the heart has frequently been described in autopsy protocols of cases of rickets. This has recently been discussed by Abt who refers to earlier reports. It seems difficult, in the absence of experimental evidence, to sustain the view that the enlargement was due to rickets per se. Similarly the muscle lesions, loss of striations, interstitial fibrosis and atrophy, are seen only in advanced cases and may be due to complicating scurvy or other conditions. They do not occur in experimental rickets.

The skeletal lesions in osteomalacia affect the pelvis, lower spine, and bones of the lower extremities. However, advanced cases show similar lesions in all parts of the skeleton. Bending, distortion and fractures commonly follow the softening. The bones cut easily, appear greatly rarefied in radiographs and are characterized histologically by an overgrowth of osteoid tissue which surrounds, in thick margins, the trabeculae and laminae and lines the haversian canals.

#### HYPERVITAMINOSIS D

The effects of excessive doses of vitamin D on the bone are premature calcification of the cartilage and active bone formations with the result that the spongiosa becomes dense and Calcium deposits also occur in various organs and in the blood vessels. Collazo and his associates studied the effects on bone in detail, and showed the late effects which are dwarfing of the growth of long bones and the production of animals with short extremities but large heads and long tails. If the dose is still greater, from ten to one hundred thousand times the minimal protective dose, rapid loss of weight and death occur. At autopsy, such animals show enormous calcium deposits in the walls of their blood vessels, and high concentrations of calcium and phosphorous in the blood serum. The metastatic calcification of the organs is largely dependent upon the amount of calcium in the diet. Indeed Harris ascribes great importance in the development of the severe effects from excessive vitamin D to the calcium phosphorous Clouse suggests that the calcium deposits are due to the inability of the kidneys to excrete the excessive amounts of calcium and phosphorous absorbed from the bowel under conditions of high mineral intake and excessive vitamin D dosage. Diseased tissues, such as tuberculous lesions and lesions of encephalitis are especially prone to calcification (Levaditi and Po).

The vascular lesions are not specific ones and Vanderveer was unable to distinguish them from those produced by certain drugs. The earliest incrustations with calcium are about the elastic fibers of the media, later the muscle fibers degenerate. Wahlgreen used smaller amounts of cod liver oil and ergosterol, and produced a chronic intoxication which affected the heart muscle. The cells were swollen, the striations indistinct, and some of the muscle fibers ruptured.

By further adjustment of the dose of vitamin D Collazo and

Kohler were able to produce nephrosclerosis which progressed to kidney insufficiency and death. Albuminuria and tubular casts were present in the urine. The larger arteries were sclerotic. Other manifestations of vitamin D intoxication of this degree are metastatic calcification, phosphaturia and polyuria as well as hypercalcemia and phosphatemia. According to Collazo and Kohler these effects may be produced at will in various species and in man.

The histologic sequences in the kidney have been reinvestigated by Goormaghtigh and Handovsky who administered calciferol at various levels to dogs. The earliest effect, according to these authors, occurs in the arteriole where smooth muscle cells and certain afibrillar cells which they believe related to the neruomuscular tissue which occurs in the heart, undergo hypertrophy. On the basis of this evidence the effect is considered to be at first stimulating. Doses in excess of 700 mg., however, produce necrosis of the smooth muscle cells. As a consequence of slightly toxic doses of calciferol and the production of reversible effects in the arteriolar smooth musculature the blood pressure rises and the response to epinephrine is exaggerated. It is significant that Goormaghtigh and Handovsky were unable to produce a permanent hypertensive effect by sustained viosterol intoxication. Once administration ceased the tissues underwent complete restitution.

The amount of vitamin D toxic for the rat has been estimated by Bills et al. as 100 times the protective dose. Such amounts gave perceptible evidence of harmful action. Four thousand times the protective dose was definitely injurious and 40,000 times strongly toxic. In terms of human doses this evidence, if directly applicable, would indicate that perceptible damage might follow 1000 times a curative dose of 3000 U.S.P. units, an amount represented by 30,000 grams of U.S.P. cod liver oil. It is apparent that the danger of

overdosage is remote when the usual preparations of vitamin D are used.

Further evidence on this point is afforded us by the experience of Reed and his associates who have used doses ranging from initial amounts containing 200,000 units to 600,000 to 1,000,000 International units per day. Under this regime toxic manifestations occurred in 9 per cent of their cases. Dreyer and Reed state: "Concentrated vitamin D is no more hazardous than many preparations used daily by physicians. To be sure there are precautions to be observed, but the early symptoms are readily recognized even by the patients themselves, and when they appear, administration of the vitamin should be discontinued at once. Usually one or two weeks is sufficient to allow before resumption of the treatment."

Bills describes the symptoms of hypervitaminosis D as follows: A sense of well being and increased appetite changing to nausea and loss of appetite; vomiting, cramps, diarrhea, and frequent urination. Sometimes neuralgia along the course of the mandibular branch of the trigeminal nerve, tenderness of gums and teeth, pain in the muscles and joints, dizziness, muscular weakness, headache, haziness of memory, and occasionally numbness and tingling in the extremities. These symptoms are usually but not invariably associated with a hypercalcemia in excess of 15 to 16 mgm. %. Discontinuance of dosage and intravenous saline administration bring prompt recovery.

The effects of prolonged administration of large amounts of vitamin D have not been as thoroughly studied. Few reports of toxic effects exist despite the widespread use of irradiated foods, sunlight lamps and vitamin D concentrates. The belief has grown in recent years that no significant hazard is associated with the use of even large amounts of this vitamin and that some of the earlier instances of toxicity were due to toxic products (toxisterol) developed by too prolonged ir-

radiation of ergosterol. No special contraindications to the use of the vitamin are known.

## SYMPTOMATOLOGY OF RICKETS

Wherever modern care of infants is available rickets disappears. Yet at the turn of the century it was the most common deficiency disease. At that time, its incidence, based on post-mortem records, was 90 per cent in the large cities of Europe. Older orthopedic surgeons can recall the horrible skeletal deformities of that time. Today such effects of dietary neglect occur only in the most benighted circles. change has been due to the regular dosing of infants with vitamin D preparations. Yet despite these recognized improvements there is still some reason to doubt that rickets prophylaxis is thoroughly adequate. Moore and his associates found evidence of mild rickets in more than 90 per cent of two large groups of children from Portland and San Diego although 80 per cent of them had received routine supplements of cod liver oil. In most the disease was relatively benign. Park, estimating present conditions from post mortem material has written: "Chronic rickets with lime salt depositions occurring sporadically is very common."

The cases most commonly seen today are very early phases of rickets. The first symptoms, head sweating, irritability and restlessness may or may not be associated with rachitic signs in the ribs. These symptoms alone commonly lead to the taking of a dietary history and the therapeutic use of cod liver oil or viosterol if such dietary measures have been neglected. The physical examination and symptoms are not diagnostically conclusive in early rickets and are of even less value in judging the progress of a case. Consequently other diagnostic means have been sought. The most valuable seem to be chemical analysis of the blood plasma.

Until recent years the most commonly employed determina-

tion was the blood phosphorus which is decreased in rickets. Calcium determinations have little value in most instances and even severe rickets may be associated with normal calcium values. Phosphorus is, however, decreased to less than 4 mgm. per cent in most cases of active rickets. It is of less value in following the progress of any given case because

TABLE 39

The Mineral Metabolism in Rickets

|         | MINERAL METABOLISM                     |                                      | CLINICAL SIGNS   |
|---------|--|--------------------------------------|--|
|         | Phosphorous balance                    | Calcium balance                      |  |
| Phase 1 | Strongly negative                      | Normal or nearly so                  | Asymptomatic or very early rickets   |
| Phase 2 | Strongly negative                      | Negative                             | Symptomatically manifest rickets recognizable radiographically                                     |
| Phase 3 | Positive (more positive than normally) | Negative                             | Clinical and radio-<br>graphic signs of re-<br>covery  |
| Phase 4 | Positive                               | Positive (more positive than normal) | Clinical evidence of<br>advanced healing.<br>Broad bands of calci-<br>fication in radio-<br>graphs |

After Rominger, E., Ergebn. d. Vitamin u. Hormonforsch., 2: 104, 1939.

treatment corrects the phosphorus content of the serum very promptly, before recovery is complete.

A superior method has been found in the measurement of the serum phosphatase, an enzyme which splits organic phosphorous compounds and liberates inorganic phosphorous. This enzyme is present in bones during growth and most concentrated at sites of most active bone formation. Its concentration has proven to be a sensitive indication of even early rickets. Moreover recovery is indicated by a slow rise of serum phosphatase and the method therefore serves as a control of the healing process. In health the phosphatase concentration lies between 8.5 and 12.5 mgm. per cent.

Morris, Stevenson, Peden and Small have tested the value of the plasma phosphatase determination in 500 cases of rickets. All values above 11 units (method of Jenner and Kay) were considered abnormal. By this standard 84 per cent of the rachitic children showed positive phosphatase tests. Parallelism was demonstrated between the height of plasma phosphatase and the severity of the process and it was thought that phosphatase values increased before other evidence of rickets appeared. However the values alone should not be accepted as pathognomonic of rickets.

A further aid in diagnosis is the roentgenographic examination of the bones, especially the wrist and forearm. A concave metaphysis with frayed margins and poorly mineralized shaft occurs in cases of moderately advanced rickets. The x-ray examination is also suited to study of the progress of the disease. The weakness of this diagnostic measure is that early, slightly developed rickets frequently cannot be recognized and that interpretation is subject to all the weaknesses of other forms of subjective observation.

Diagnosis is not difficult if the case be more advanced. The rachitic rosary, the tumefaction of the junction of cartilage and bone of the ribs due to the overgrowth of osteoid tissue and cartilage, may be seen or felt, the anterior fontanel will be found to be wider than normal and areas of softening may be detected behind the ears.

The advanced case is so striking in appearance it may be identified at a glance. The head is squarish in shape due to excess osteoid tissue forming beneath the periosteum of the skull. The rosary pattern is often exaggerated by depression or extension of the sternum and the formation of Harrison's



PLATE XXXVIII. Rachitic deformity in an adolescent. (Photograph from collection of Dr. Win Watters.)



groove. Spinal curvatures, deformities of the leg bones, etc., and delayed dentition are other features.

The highest incidence of the disease occurs late in the first year of life but it may occur earlier or much later. Maxwell and Wolfe have both reported series of cases of foetal rickets with characteristic lesions in both bones and teeth. The infrequency of such cases had previously led many to consider the fetus immune to rickets. Rickets also occurs, but infrequently, during puberty.

Vitamin D deficiency is a constitutional disturbance and more than the skeletal system is affected. Less attention has been paid to other manifestations of the deficiency than to the skeletal changes but they are helpful in recognizing the presence of the disease and their investigation may prove to be very informative. Rachitic children are usually fat and weak. Muscle weakness is evident in the lax abdominal muscles and probably in the constipation which so often accompanies rickets. Anemia is present in many cases but is due to other causes than vitamin D deficiency since it does not occur in experimental rickets and when it is present in human cases can be explained on other grounds.

Osteomalacia is most common during the period of life associated with childbearing and affects chiefly women who have repeatedly been pregnant or who are lactating. The earliest symptom is usually pain, especially in the back and sacral region. Weakness is associated and sometimes stiffness and contractures of the limbs. The muscular weakness is said to be pronounced in the adductor muscles of the thighs. If deformity follows it is first observed in the same parts in which pain occurs, namely the lower spine and pelvis. More than half of the recorded cases are said to have shown signs of tetany which is easily understood when the excessive demineralization which occurs in osteomalacia is appreciated. The loss of lime salts has been estimated to be as much as two thirds of the normal supply.

# THE TREATMENT AND PREVENTION OF RICKETS AND RELATED DISEASES

The prophylaxis and treatment of rickets requires an adequate dietary intake of calcium, phosphorus, and vitamin D. The requirements of the first two are more definitely known than that of vitamin D. This is largely true because constitutional factors, the invisible intake of vitamin D through skin irradiation and the differences in efficiency of different vitamin preparations have tended to confuse the meaning of clinical trials.

Some years ago the Council of Pharmacy of the American Medical Association recommended one to two teaspoonfuls of cod liver oil daily for the prevention of rickets and three teaspoonfuls for the treatment of rickets. The recommendation was a wise one. However the average concentration of vitamin D in the better cod liver oil preparations has since doubled. Nevertheless the recommendation is still in line with present belief that the older recommendation was less than optimal. Hess at one time recommended 1100 International U.S.P. units daily. This is the same dosage recommended by Shelling and Hopper in their study of viosterol and approximately twice as much as various clinicians have found effective in most cases. It is well known, however, that premature infants and very rapidly growing ones require 2 or 3 times the usual dose.

For the treatment of rickets the level of dosage may be 1000 units to many times as much. The larger amounts induce more rapid healing.

Patients who do not respond to smaller doses require larger amounts. We have already referred to Albright, Butler and Bloomberg's study of vitamin resistant cases. Doses of from 150,000 to 1,500,000 units were sometimes necessary. In diseases such as renal rickets no response has been reported from any amount of vitamin D and Shelling and Hopper con-

sidered vitamin D contraindicated since a tendency to metastatic calcification is frequently present in such patients.

Vollmer recommends, as treatment for neonatal and infantile tetany, severe rickets, rickets complicated by whooping cough, pneumonia or any chronic infection and as treatment of other cases of rickets when there is reason to believe the routine administration of vitamin D will not be carried out, the use of a single large dose of vitamin. His plan is to give 600,000 units by mouth. The dose is mixed with one or more feedings. Amounts less than 200,000 units do not regularly produce a favorable response but doses between 200,000 and 400,000 units are usually sufficiently large for young infants. Nádrai gave similar amounts but administered them intramuscularly.

The use of massive doses of vitamin D has generally been found safe, at any rate harmful effects have not been recognized. In the use of the vitamin in unrelated conditions such amounts may justifiably be given under supervision and without exceptional precautions. In the case of the single doses Vollmer has used, toxicity is not encountered. But, as Park points out: "In rickets the object is to restore normal calcification to the skeleton. The physician does not need to induce toxic action to bring this to pass and seeks to avoid even an approach to it." Park's recommendation is 1,200 units daily which brings the majority of cases under control within three weeks. If larger doses are required concentrated sources are used and the dose increased to the point of effectiveness. When the rachitic process has been reversed the dosage is reduced but not to the usual, low preventive level. This is necessary because 6 months is often needed for complete cure. Rachitic infants frequently manifest what may be called a rachitic constitution and this requires prolonged treatment. Jeans writes that babies who get around 400 units of vitamin D daily grow at a greater rate than those who get more. Those who get 1500 units or more "have a poorer appetite and the growth response is less."

Shohl cured 2 cases of rickets by administering large amounts of citrates. A mixture of 20 cc. of molar citric acid and 30 cc. of molar sodium citrate was added to the formula. This corresponds to the amount of citrate in 5 or 6 large oranges. Recovery was evidenced chemically and radiographically.

The mechanical treatment of deformities is best undertaken at the earliest possible opportunity.

### ATYPICAL FORMS OF VITAMIN D DEFICIENCY

The significance of subclinical vitamin D deficiency rests on observations of increased requirements of pregnant animals and the effect of deprivation on the teeth. There are two sides to the latter problem, the effect of deficiency on the structure of developing teeth and the effect on maintaining the resistance to caries of teeth already formed.

The requirements of pregnancy and of normal tooth structure are related since rachitic lesions in the milk teeth occur before birth. For a time it was believed that the fetus was immune to rickets. This seems a rather surprising point of view since osteomalacia is commonly related to pregnancy and the combined requirements of mother and fetus are naturally greater than those of the mother alone. At any rate undeniable evidence of rickets during intrauterine life now exists, including rachitic disease of the teeth.

Maxwell and Hu reported three cases of fetal rickets and Maxwell has since collected fourteen more. In a typical instance the mother showed osteomalacic lesions of her pelvis, the infant was born with typical rachitic rosary and Harrison's groove. The anatomic evidence is thoroughly satisfactory. Wolfe has reported three similar cases and has described the lesions of the teeth. The location of the defective, hypoplastic zones of enamel and dentin were such that Wolfe could, on the basis of the established sequences in the maturation of

the deciduous teeth, determine the period of intrauterine life in which the rickets occurred. Histologic examination showed the predentinal zone to be widened and the dentin unevenly stained and with irregular margins. Wolfe's study is particularly instructive for it shows that deciduous teeth stigmatized by fetal rickets do not recover their normal structure with recovery from the rickets. These lesions are indelible, while the skeletal manifestations may entirely disappear.

This observation is of importance to the problem of defective teeth. Dick had found no deciduous incisors which were grossly hypoplastic and concluded that the fetus was therefore immune to rickets. But Mellanby found minute defects in 80 per cent of the deciduous teeth she examined. It is essential to her theory that either rickets may occur and disappear, leaving only dental lesions as permanent residua, for that degrees of rickets exist which are unrecognizable by our present means of examination but which are capable of interfering with the normal development of the teeth. Wolfe's studies establish that the first hypothesis may be true. That the second theory is also true is at least strongly suggested by the frequency of defective permanent teeth, formed during post natal life when the incidence of clinical rickets is a matter of record.

Infantile rickets occurs during the period when the permanent teeth are being formed, between the fourth month and the second year of life. The effects of rickets are classified according to the pattern of the lesions. Basically all are the same as they represent areas of hypoplasia. The lesions may be in the form of wavy enamel, point or dimple defects, horizontal furrows or complete deficiency of the enamel of the crown (Freudenberg). In Mellanby's extensive examination of the teeth of British children she found only one-fifth of 1260 teeth were normal, the remainder having hypoplastic lesions of various degrees from 17.5 per cent which were slightly hypoplastic and 25.9 per cent moderately hypoplastic

to 35.4 per cent very hypoplastic. The significance of this common developmental defect was not difficult to find. Ninety per cent of the poorly developed teeth were also carious while ninety per cent of the normal teeth were free of caries. The ten per cent discrepancy, according to Mellanby, is due to the effect of diet subsequent to the formation of the teeth.

The association between rickets producing diets during pregnancy and rickets in the offspring has been repeatedly demonstrated in experimental studies. Hess and Blackberg in 1923 observed a varied susceptibility to rickets depending on the diet of pregnant animals. Extensive work was reported by Toverud and Guttorm who had observed a negative calcium balance during pregnancy and were able to reproduce the circumstances in dogs. The offspring had defective teeth. By varying the time when the bitches were fed the rachitogenic diet they were able to regulate the interval post partum when the young animals developed rickets. If the poor diet was fed throughout pregnancy the pups developed rickets three weeks after delivery. If the poor diet was fed only during the period of lactation the pups developed rickets six weeks after birth. If the diet was adequate throughout pregnancy and lactation the pups required thirteen weeks feeding of a rickets producing diet before they developed the disease. Similar results were reported by Grant and Goettsch in rat experiments in which the measure of the mother's reserves of antirachitic substance was the number of litters of rickets resistant young she could gestate.

All of these results must be interpreted in the light of the various factors involved in rickets rather than as expressions of vitamin D deficiency alone. The high calcium excretion in milk plays a prominent part and in the rat at least this effect seems only partially related to the vitamin D supply (Kletzien, Templen, Steenbock and Thomas). These authors

found the calcium content of newly born rats to be fairly constant, irrespective of the vitamin D given the mother.

More specific information on the transmission of vitamin D via the placenta has recently been furnished by Toverud and Ender who assayed the livers of still born infants. Among forty-four cases about half were found devoid of vitamin D, small amounts were present in fifteen and "abundant" quantities in five. The latter group were infants whose mothers had been receiving considerable amounts of the vitamin during pregnancy. There would seem to be ample justification for the liberal use of vitamin D and high mineral diets during pregnancy and lactation. A commission of experts has recommended 340 Int. units of vitamin D daily under such conditions.

The very nature of the rachitic lesions predisposes to the misplacement of nests of cartilage cells. This may lead to cartilaginous tumors later in life. While this is not a manifestation of subclinical rickets it forms an interesting example of the ramifications of the deficiency diseases. Virchow first suggested that rachitic inclusions might lead to the subsequent formation of enchondromata and numerous other pathologists have similarly interpreted certain benign cartilaginous tumors. The subject has recently been discussed by McMaster.

Vitamin D has been used empirically in various conditions not related to rickets, psoriasis, arthritis, hay fever, hemorrhage associated with jaundice, the toxemia of pregnancy and myopia. It has been used more understandably in osteogenesis imperfecta (without results—Hansen, McQuarrie and Ziegler). These reports are outside the scope of this book. It is believed, nevertheless, that other, uncommon disturbances of mineralization will eventually be recognized which may be benefited by vitamin D therapy. We have observed cases in middle aged and elderly individuals in which symptoms and lesions were suggestive of vitamin D deficiency.

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## CHAPTER XIX

# THE NATURE AND FUNCTION OF VITAMIN E

Evans and his coworkers in 1921–22 first produced satisfactory evidence that reproduction is affected by a fat soluble factor different from vitamins A or D. Using Osborne and Mendel's basal diet for rats (casein 18; cornstarch 54; lard 15; butter fat 9; salt mixture 4; plus 0.4 to 0.5 gram dried yeast daily) they found that female rats put on this diet appeared in every way normal, had normal estrus, normal ovulation, normal implantation of eggs after fertilization but failed to produce normal litters. The expected young were not born but resorbed.

Supplementing with vitamins A, B, and D did not prevent fetal death but certain foods proved protective, viz., lettuce, whole wheat, wheat germ, and dry cereals. Consequently in 1922, Evans and Bishop postulated the existence of a new vitamin, calling it for the time being substance "X". In 1923 they reported that its lack not only produced fetal death in female rats but complete sterility in male rats.

The existence of this vitamin was further confirmed by Sure who suggested that it be designated vitamin E.

The early history of the vitamin has been reviewed by Evans and more recently summarized by Mattill who with his collaborators was a major investigator in developing the properties of this vitamin.

### HOW VITAMIN E POTENCY IS EXPRESSED

Evans defines as a unit of vitamin E the amount of source necessary in daily doses during the 21 days of gestation to insure the production of healthy young. He usually expresses

potency in terms of the total amount required, e.g., an Evans 525 mgm. wheat germ oil is one which when fed in 25 mgm. doses per day for the 21 days of gestation prevents failure to give birth to a live litter.

Pacini and Linn have suggested another type of unit. Like Evans, they first determine the minimal amount per day for 21 days necessary to secure normal young. They suggest as the unit 1 gram divided by this amount in milligrams. Thus if 25 mgm. was the minimum daily dose of what Evans calls a 525 mgm. oil; by the Pacini-Linn system that would be a 40 unit oil (1000/25 = 40).

TABLE 40

| OILS       | PER CENT TOCO-<br>PHEROL IN NON-<br>SAP. FRACTION | IN WHOLE OIL | ACETYL NUMBER |
|------------|---|--------------|---------------|
|            | per cent  | per cent     |               |
| Wheat germ | 13.4  | 0.52         | 14.4          |
| Maize germ |   |              | 11.0-11.5     |
| Lettuce    | 4.3   |              |               |
| Linseed    | 2.34  | 0.023        | 4.0           |
| Olive      | 0.935   | 0.008        | 10.6          |
| Sesame     | 0.63  | 0.005        |               |
| Palm       | 0.55  | 0.0027       | 1.9-8.4       |

These unit values are of course obtained by bioassay method but several chemical tests have been suggested that give promise.

The acetyl value of an oil is a measure of its hydroxyl group content. We have already noted that the vitamin E molecule contains one hydroxy group that can be acetylated. The acetyl number of an oil is therefore a rough approximation to its vitamin E content. Mattill has shown that it may be used to indicate potential E values though obviously the presence of OH groups in other than the vitamin E constituents of the oil makes this non-specific for E. See table 40.

As stated above, several chemical methods of estimating alphatocopherol content have been suggested.

A colorimetric method was devised by Emmerie and Engel. It is based on the fact that vitamin E is destroyed by an oxidant such as ferric chloride with the reduction of the iron and the fact that the  $\alpha$ -dipyridil reagent measures only non-reduced iron. Unfortunately there are other substances in oils that can reduce iron beside vitamin E which must be eliminated to make this test specific. Emmerie and Engel state that an adsorbent which they call Floridin XS earth will after repeated treatment with HCl on a hot water bath adsorb carotenoids and vitamin A in benzine solution and adsorbs little or none of the x-tocopherol.

Furter and Meyer claim that when a-tocopherol is treated with concentrated HNO<sub>3</sub> in absolute alcohol with short heating, an intensely red colored substance (probably an oxonium salt by addition of HNO<sub>3</sub> to the tocopherolquinone formed by oxidative cleavage) develops and makes possible estimation of tocopherol content by photometer. They claim this color is specific for tocopherol and has a maximum absorption band at 467 m  $\mu$ . It is applicable to oils without saponification.

Karrer et al. report it possible to estimate tocopherol potency by potentiometric titration with gold chloride—2 mols. AuCl<sub>3</sub> equivalent to 3 mols. of B-tocopherol. Using this method they report the values for oils given in table 40. In this table we have also given the acetyl values of these same oils illustrating the value of these numbers as possible indices of vitamin E value.

## PROPERTIES OF VITAMIN E

Evans and others have studied the behavior of the vitamin in natural products and have noted the following characteristics:

The vitamin is stable at high temperatures—up to 250°C.—in dry condition.

Aeration at 97°C. does not destroy it but the presence of rancid fat is very destructive, hence aeration at high temperatures may first induce such

rancidity in fats in which the vitamin is dissolved and thus, indirectly, bring about the destruction of the vitamin itself.

Vitamin E is stable to ordinary light but prolonged ultra-violet light slowly inactivates it. It is often stable to saponification at 35°C. or in boiling alcohol. On the other hand, there is sometimes as much as 75 per cent destruction during saponification which may be avoided by using methyl alcohol or by adding an antioxidant such as dibenzylamine. Weber et al. report the activity of E destroyed by contact with rancid fats such as those made rancid by aeration, by heating in the presence of oxygen, or by treatment with ozone or falmitic peroxide. They report no destruction by fats heated under N-reflux, nor with fats to which acrolein, allyl alcohol, or straight chain aldehydes and ketones were added.

Vitamin E is not destroyed by hydrogenation but is by bromination.

Cooking or drying by steam distillation is without harmful effect.

Certain vigorous oxidants such as permanganate and perbenzoic acids cause destruction.

No toxic effects have been observed with large doses of the vitamin.

#### THE FUNCTION OF VITAMIN E

In rats vitamin E deficiency has the following effects:

Male rats become infertile through degeneration of the germinal epithelium and the damage once incurred cannot be repaired by feeding of vitamin E.

Female rats deprived of vitamin E fail to carry their young to parturition; the embryos are damaged, die and are resorbed, but the reproductive mechanism itself is not damaged since adequate dosage restores fertility and ability to produce normal litters. Lack of E therefore produces fetal death, not destruction of ability to conceive.

These are the two major effects of vitamin E deficiency and on these are based bioassay methods for establishing vitamin E potency. Their anatomical features are discussed in Chapter XX.

Other functions for the vitamin have been suggested but at present we lack conclusive evidence on these values.

Of its need by man, Evans says:

Indubitable as are the results which have been obtained by the careful study of small rodents—rats and mice—one cannot say that equally conclusive proof of the need for vitamin E on the part of other animal forms has yet been furnished.

At the present writing neither the U.S. Food and Drug Administration or the Council of Pharmacy of the American Medical Association permits therapeutic claims for this vitamin in label declarations.

One reason for this present attitude probably lies in our lack of knowledge of the distribution of vitamin E in natural products. Qualitatively we know it is widely distributed in foods but quantitatively data are meager. There have been claims made for its value in certain conditions which we may review briefly.

## HABITUAL ABORTION

Vogt-Møller by subcutaneous injection of a wheat germ oil preparation (Fertilan) induced pregnancy in 35 out of 50 cows that had repeatedly failed to deliver normal young. He also reported (1933–36) that administration of 3 grams of wheat germ oil daily resulted in the birth of living children in 17 out of 20 habitual abortion cases in one series and 39 out of 50 in another series.

Watson reported success of wheat germ oil treatment in 34 out of 46 cases of previous abortion and Currie by administering daily, from time of first attendance to the onset of labor, a concentrate made from 5 gms. wheat germ oil secured 23 normal births out of 24 cases whose previous 73 pregnancies had given only 14 per cent of living children.

In an address on the diagnosis and treatment of Vitamin Deficiencies, Dr. L. F. Barker (April, 1939) comments as follows:

Several reports of normal pregnancies after the use of vitamin E (wheat germ oil) in women who had earlier had successive miscarriages have recently been made, but further observations are necessary before we dare speak positively about the significance of vitamin E deficiency as a common cause of habitual abortion in women. The dosage of wheat germ oil in patients who have had earlier, successive miscarriages is from 3 to 6 cc. given by mouth as soon as pregnancy is recognized. This dosage may be continued through pregnancy and if at any time abortion be threatened larger doses

(up to 20 cc. daily) may be given. Instead of wheat germ oil, wheat germ itself may be given daily, one ounce being regarded as a liberal dose.

Shute noted that the blood serum of aborting women had an increased resistance to tryptic proteolysis and that rats four months on a vitamin E-deficient diet also developed this property in the serum. Normal digestion was restored by vitamin E administration.

#### VITAMIN E AND HEMATOPOIESIS

Pergallo and Fiori report that administration of vitamin E increases both the erythrocyte and hemoglobin count in dogs. This work needs confirmation.

#### VITAMIN E AND MUSCULAR DYSTROPHY

This condition, first described in herbivorous animals by Goettsch and Pappenheimer, was produced in guinea pigs and rabbits, even in utero, by a diet deficient in E. Rats on the same diet did not manifest the disease. It is quite probable here, that it is the antioxidant effect of the vitamin that is involved and its preventive action against the behavior of auto-oxidizable fatty acids. For discussion of the problem see Chapter XX.

#### VITAMIN E AND THE ENDOCRINES

Verzar suggested that vitamin E might affect pituitary response. His theory was that vitamin E acted like anterior hypophyseal hormone in producing precocious sexual maturity in young female rats but not in castrates and that vitamin E might be a precursor of the hypophyseal hormone. This viewpoint has not received satisfactory confirmation and to date the relation of E to hormone formation is still a theory requiring substantiation.

### VITAMIN E AND GROWTH

Juhasz-Schäffer showed that vitamin E accelerates cell growth in tissue cultures. It has been frequently reported

(Schioppa) that vitamin E increases the number and viability of the young. Here again we may be dealing with a result of the anti-oxidant property of vitamin E. Hickman has suggested that vitamins A, B<sub>1</sub>, B<sub>2</sub>, C, have all been found parts of an oxidizing system and that perhaps the anti-oxidant action of E is necessary to form a part of a control or protective system in combination with these factors. Topical application of E as of benefit in wound healing has been reported by Pagreffi and by Leranth and Laszlo.

#### SUMMARY

More clinical and experimental evidence and availability of vitamin E in pure form is needed to establish its rôle in human physiology and pathology and its therapeutic value.

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## CHAPTER XX

## VITAMIN E DEFICIENCY

The effects of vitamin E deficiency have been determined mainly by experiments on rats. The functional result observed is the production of sterility in both sexes and hence the name 'anti-sterility' vitamin. The term is to a degree misleading, for other pathological results occur which are in no way related to fertility. It seems evident now that the association of vitamin E and sterility is due to the heightened cellular activity in the fetus and testis and that other tissues, such as the cerebral cortex, also require the vitamin during their period of rapid growth.

The functional effects of vitamin E deficiency in the male appear after four months' depletion and develop through four

stages. The first stage consists of a period when normal sex responses are present as well as normal appearing sperm cells but the sperm lacks the power to fertilize the female ova. Later, sperm cells are entirely lacking from the bouchon; thirdly, loss of power to form the bouchon appears and finally

the animal loses sex interest as well.

The germinal tissue of the female is not affected and both ova and follicles appear perfectly natural. These tissues are functionally active. A depleted female conceives normally

but the fetuses invariably die.

Placental tissue, implantation site, and fetal tissues are all affected in vitamin E deficiency. The implantation site is stigmatized by the presence of many free erythrocytes and hemorrhagic lesions also occur in the placenta. The sinusoids are tremendously distended. The fetal effects are predomi-

nantly limited to the hematopoietic and mesodermal tissues. Probably, in part at least through the retarded growth of the blood islands and the resultant tissue starvation, the general development of the fetus is reduced. At any rate, whether through asphyxia or otherwise, the products of conception die and are slowly resorbed.

The third functional effect of vitamin E deficiency is seen in the offspring of depleted mothers. Under such circumstances and at about three weeks post partum a paralysis develops which resembles an upper motor neurone lesion. This appears in some, but not in all the offspring of a particular mother, exhibiting, in common with most of the other manifestations of vitamin E deficiency, a strong tendency to irregularity.

Another striking characteristic of vitamin E deficiency is that the effects are largely irreversible. Recovery in the male, if it occurs at all, requires nearly a year of treatment and after the fifth day of gestation the litter of a depleted mother cannot be saved by vitamin E dosing. The nervous lesions are more amenable to treatment but in this case, as in the testis, the assumption is that cells not affected by the deficiency are responsible for the subsequent growth. The tendency to irreversibility is partly overcome by the large doses which the isolation of vitamin E have made possible. In studies of this kind Evans and Emerson observed that whereas 3 mgm. of  $\alpha$ -tocopherol is sufficient to cure sterility in young female rats, 2 or 3 times as much is required at the 8th month of life and 8 to 10 times at the close of the first year. Older females conceive and implant but the young cannot be rescued by any practicable dose level of vitamin.

## ANATOMIC EFFECT

The anatomic results of vitamin E deficiency are seen to best advantage in the testis. The lesions have been divided into five stages, though various stages are often simultaneously



PLATE XXXIX. Two seminiferous tubules from rats fed a diet deficient in vitamin E. The cells have atrophied and desquamated. The crescentric chromatin masses and huge, multinucleated cells are typical of advanced vitamin E deficiency.



present in different parts of the same organ. The first stage is marked by fusion of the mature or maturing spermatozoa and lysis of their chromatin. At about the same time or very soon thereafter secondary spermatocytes and spermatids also show chromolysis. Their nuclei become crescentric (2nd stage). In the third stage these cells fuse into large and numerous giant cells containing many crescentric or bead-like nuclei, often arranged around the periphery of the cells. In the fourth stage the same process affects more immature cells—primary spermatocytes and spermatogonia.

The entire process usually requires 35–50 days (Mason) and the first changes are evident after a depletion period of from 50–100 days. As the cells degenerate they slough off and disappear leaving the terminal stage in which a thin rim of immature germinal cells alone remains. This stage also occurs in animals depleted of vitamin A so is hardly characteristic of

vitamin E deficiency.

The work of numerous investigators shows that the first evidence of structural abnormality appears after a period in which the sperm cells appear to be natural but are functionally inactive. For a fuller discussion of the histopathology of vitamin E deficiency the reader may consult the reports of Evans and Burr, Kudrjaschov, Mason, and Juhasz-Schäffer.

Degeneration of the testis occurs in various other vitamin deficiencies as well as in inanition. In none of these conditions do the characteristic changes just described occur. A close similarity between the lesions in vitamin A and E deficiencies is due to the frequent combination of the two and if adequate vitamin E is supplied, the testes from the A deficient animals may be readily distinguished from those of E deficiency.

In A deficiency the germinal cells slough off at an abnormal rate and the denuded seminiferous tubules gradually shrink in size. Some maturation continues in the deeper layers of the germinal cells, probably at a greatly reduced rate. One other typical result of vitamin A deficiency is said to be the persistence of nests of maturing cells. However, in prolonged and severe deficiency a stage of atrophy is reached which is indistinguishable from the fifth stage of vitamin E deficiency.

In common with most other avitaminoses the lesions of vitamin A deficiency are rapidly reversible and in experimental studies this serves as a valuable differentiation between these less specific effects and the results of a deficiency of vitamin E. In inanition, as in other vitamin deficiencies, sloughing of the germinal epithelium is less pronounced than in  $\Lambda$  deficiency. The end stage of the atrophy of inanition resembles the uncompleted seminiferous tubules seen in immature animals.

While vitamin A and E effect the testis in different fashion, Mason has found a relationship between the action of the two. The lesions of vitamin E deficiency may be retarded if vitamin A is also lacking. Mason's explanation of this phenomenon is that the maturation rate is retarded by the deficiency and less vitamin E is needed to insure natural nuclear function and structure.

Associated with the permanent sterility of prolonged depletion are lesions of the uterus and ovaries. The former becomes yellow-brown in color (brown atrophy) and the ovaries contain large corpora lutea. Patchy areas of fatty degeneration may be found in the musculature. Virgin rats develop these lesions as well as animals which have undergone a pathological pregnancy with resorption. Vaginal bleeding and fibromyomata are sometimes seen (Barrie).

## MUSCLE DYSTROPHY AND VITAMIN E

An early observation of vitamin E deficiency was the presence of paralysis in the suckling young of depleted rats. Evans and Burr found this to be a regular manifestation before the 25th day of life. In some an initial paretic stage was seen but in most spasticity was the first sign. The spasticity was limited to the hind quarters and eventually was associated by

symmetric baldness and muscular atrophy. Early treatment with vitamin E was sometimes curative.

Similar lesions can be produced in adult rats (Ringsted) but require 5 months or longer of a deficient diet. The symptoms commence with dragging of the legs, slight incoördination and some thinning of the hair. The adductor muscles weaken and the gait becomes straddling in type. Finally the hind quarters are dragged about and cannot be adducted.

More extensive but otherwise indistinguishable lesions occur in herbivora on diets deficient in vitamin E. They were first reported by Goettsch and Pappenheimer in guinea pigs and rabbits. The original premise was that they were due to vitamin E deficiency because the diets were recognized as deficient in that factor. Subsequent experiments failed to verify this theory and for a time an unknown dietary factor was postulated.

In this case, as in the rats, the lesions are those of hyaline or waxy degeneration and are apparently similar to those illustrated and described in Chapter XVI. Calcified masses occur. The hemorrhages are not extensive and it is possible they could be distinguished from those due to scurvy by this difference. Similar, if not identical lesions, are common in other apparently unrelated dietary deficiencies. In addition to scurvy and beriberi, previously mentioned, they have been reported in rickets (in rats) by Kihn and in fat deficiency by Borst (quoted by Kihn).

In the herbivora the lesions appear abruptly after a protracted deficiency but they may be hastened by adding cod liver oil to the diet. Madsen, McCay and Maynard first thought this was due to a toxic agent carried by the oil since hydrogenation (which stabilizes fats which easily become rancid) largely destroyed this action of cod liver oil and vitamin A and D concentrates delayed the appearance of the lesions. But Morgulis and his colleagues demonstrated that two factors must be lacking in the diet to produce dystrophy.

One of these was first suspected to be vitamin E since it occurred in the natural sources of the vitamin and in the non-saponifiable portion of their fat-soluble fraction. The other was discovered to be water soluble and was found in yeast and certain B concentrates as well as the water-soluble fraction. The acetone extractable material of wheat germ contains both fractions.

This explanation has been tested and confirmed and the fatsoluble agent proven to be alpha tocopherol (Mackenzie and McCollum, Shimotori, Emerson and Evans). The nature of the second fraction is still unknown. It has been suggested it may be vitamin B<sub>4</sub>.

Verzar reports that creatinuria is regularly associated with muscle dystrophy in rats and responds very rapidly to treatment with dl- $\alpha$ -tocopherol. In a single day the excretion of creatin decreased three-fourths and the ratio of creatin to creatinine from 40 to 130 per cent to about 5 per cent. The water and chloride content of the muscle is increased. Knowlton, Hines and Brinkhous followed these values as well as the creatinine content and found water and chloride content to increase before weakness was manifest. Synthetic  $\alpha$ -tocopherol controlled all of these signs.

An important clue to the pathogenesis of muscle dystrophy is furnished by a recent report by Pappenheimer and Goettsch. It was discovered that section of the sciatic nerve, if performed before the 18th day of life, completely protects the muscles on the same side from dystrophic changes. Removal of nerve impulses prevented lesions.

As has been mentioned the muscle lesions were originally observed only in young animals. Mackenzie, Mackenzie and McCollum succeeded in producing identical lesions in rats which had been on a normal diet until full grown. The time necessary for the production of the dystrophy was 8 to 10 months. The first symptom was spreading of the hind legs and lowered posterior abdomen when walking. After 45 to

50 weeks extreme abduction of the hind legs was seen and finally the hind legs became lifeless. Tremors and incoördination of the forelegs and head were noted shortly afterward and became so severe they interfered with eating. Excitement and noises intensified the tremors and even induced convulsions and coma. The muscle lesions were characteristic. Therapy was without effect.

Thus the two important factors in vitamin E deficiency production are duration of the depletion and body store of vitamin at the beginning of the experiment. Since depletion is slow and the requirements not large great variations are possible. Mason and Bryan have discussed this as it applies to the biological assay of vitamin E sources. A deficient diet during the last half of lactation minimizes the reserves of the off-spring. Testicular degeneration can be hastened by this means and lesions occur coincidentally with the first appearance of spermatozoa.

Vitamin E deficiency results in morphological changes in the anterior lobe of the hypophysis where enlarged, vacuolated basophilic cells (castration cells) appear according to van Wagenen. Verzar suggests that the retarded growth, lowered basal metabolism and inferior fur of deficient rats are due to malfunction of the hypophysis and that the vitamin may be necessary to the formation of the hormone. These and other signs of deficiency cannot be explained on the theory of retarded nuclear division.

The apparent relationship between vitamin E and nuclear division has prompted studies of the rôle of the vitamin in carcinogenesis. Davidson reports that mice on a high vitamin E intake are resistant to coal tar carcinoma. Adamstone said that chicks on a deficient diet showed "effects apparently due to the phenomenon of uncontrolled and unrestricted cell growth, simulating malignancy." Rowntree, Lansburg and Steinberg claimed to have produced malignant tumors in rats by wheat germ oil extracts. This has not been confirmed. Negative

results were reported by Evans and Emerson, Carruthers and others.

Vitamin E apparently acts directly upon the tissues affected and is consumed during cellular activity. Juhasz-Schäffer found it accelerated the growth of tissue cultures. In rat experiments in which the litter size and number is increased by increasing levels of vitamin B feeding the consumption of vitamin E seems to be accelerated for the frequency of paralysis among the young is increased (Evans and Burr).

Species immunity has not been extensively investigated. Thomas and associates have found the goat entirely independent of a vitamin E intake. Three filial generations were maintained in good health on a deficient diet. Their muscle and milk were free of vitamin E although animals on a natural diet have vitamin in both places. Anderson, Elvehjem and Gonce describe weakness in the extremities of the offspring of dogs fed evaporated milk, cod liver oil and minerals from weaning. There was little muscle tone, the animals were hypersensitive. Loss of fur was observed, the skin became dry. The condition resembled cretinism. Typical muscle lesions are said to have been present. Male puppies were more severely diseased than female ones. The positive results in these experiments (and the previously reported failures to produce deficiency effect in dogs) were ascribed to the combination of dietary depletion and pregnancy.

### RELATION TO VITAL FUNCTIONS

Vitamin E appears essential to certain vital functions associated with rapid cellular multiplication rather than to a particular tissue, as is true of vitamins C and A. Juhasz-Schäffer and Mason, both on the basis of extended study of the histologic effects of vitamin E deficiency, associate the vitamin with cellular division.

Mason considers that the nucleus is affected because the damage is irreversible, suggesting a more central and funda-

mental damage than would be the case if the cytoplasm alone were affected and because the first visual evidence of the deficiency is in the structure of the nucleus.

This theory would seem to explain the major effects of vitamin E deficiency. The application to the testis and fetus is obvious. In both cases cellular division is extremely rapid. The nervous lesions are not dissimilar. The paralysis is closely associated with the weaning period which is also the time of greatest nuclear activity in the cerebral cortex of the rat. A similar opinion was reached by Adamstone and Card from histologic study of the testes of fowl. Adamstone has also followed the sequences in the egg embryo and ascribes fetal death to damage to the blastoderm, the vessels of which are destroyed and cause the death of the embryo by starvation and/or anoxemia.

## IS VITAMIN E NEEDED BY MAN?

Two types of evidence have been reported suggesting that vitamin E deficiency occurs in man. Mason sought for lesions in human material similar to those seen in rats. In one human testis he found histologic changes similar to those associated with the third stage of vitamin E deficiency. Older reports were also reviewed, including one by diBiasi who described rather similar lesions in 40 per cent of a large series of necropsies and in 57.5 per cent of 80 cases thoroughly searched for such lesions.

Early reports of the cure of habitual abortion (Vogt-Møller, Poulsson) have been confirmed in later papers. Vogt-Møller reported that more than half of 72 women who habitually aborted were able to deliver term infants after treatment with wheat germ oil. Watson and Tew reported success in 34 of 46 treated cases. Success was claimed by Currie and Cromer. Non-fertile women are not benefitted. Shute has used wheat germ oil in the treatment of abruptio placentae and menopausal vulvovaginitis. The latter condition was relieved in

all cases although one patient developed urticaria lesions as a result of the treatment.

### USE OF VITAMIN E IN OTHER CONDITIONS

Ringsted's description of the effects of vitamin E deficiency in rats has prompted the use of the substance in amyotrophic lateral sclerosis and muscular dystrophies. Wechsler administered 9 to 18 mgm. of a-tocopherol acetate daily to 2 cases of amyotrophic lateral sclerosis with immediate and continuing improvement. Bicknell reported success in 2 of 4 treated cases. He also treated 26 cases of muscular dystrophy with considerable success. The vitamin was given as dried wheat germ. Wechsler was unable to accomplish much in far advanced cases. Vitamin E has also been used in various other diseases with varying results. In certain instances effects have been secured only through the use of larger amounts and doses of 150 to 250 mgm. daily are not excessive. Maintenance doses of 50 mgm. have been recommended but this varies between individuals and should be adjusted to the particular patient.

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## CHAPTER XXI

# THE NATURE AND FUNCTION OF VITAMIN K

In 1931, McFarlane and associates in Canada noted that chicks fed on ether extracted fish meal suffered a high mortality and extensive bleeding from small wounds. They also noted that the blood of these chicks failed to clot, even after hours of standing.

In the years just preceding Dam (1929–30), while studying fat metabolism in Copenhagen, noted marked hemorrhages and stomach changes in chicks on a special diet. He and his associates (1934–35) followed up these observations and in 1935 postulated the existence of a fat-soluble vitamin whose deficiency was causative of the condition; a vitamin demonstrably different from vitamins A, D, or E. Dam proposed for this vitamin the name "Koagulation Factor" or vitamin K.

There are various theories of blood clotting but general agreement that the process proceeds by two steps:

Step 1. Prothrombin + thromboplastin + ionized calcium forms thrombin.

Step 2. Fibringen + the enzyme thrombin produces the fibrin clot.

In brief, the conversion of serum soluble fibrinogen to the solid fibrin requires the action of the enzyme thrombin. This enzyme does not exist in the circulating blood but is formed from a precursor (prothrombin) present in the blood which is converted into the enzyme thrombin by combining with a phospholipid of the blood platelets (thromboplastin or cephalin) and ionized calcium.

On this basis it is evident that failure of blood to clot can result from insufficiency of any one of the three factors of step 1, but there was evidence that in the phenomena observed by Dam and by McFarlane the factor deficient in the clotting system was prothrombin and that for the manufacture of prothrombin vitamin K was essential.

Dam and Schønheyder worked out a method of measuring clotting time in chicks as a means of estimating vitamin K potency of various sources. Using this method they arrived at the following definition of a vitamin K unit:

The amount of vitamin which is required per gram of the animal on three successive days in order to render the clotting time of the blood normal.

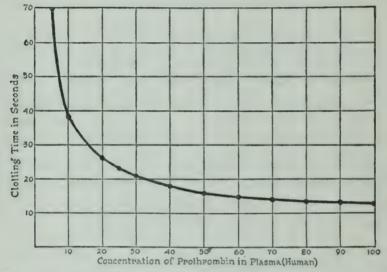


Fig. 27. Quick's figure for estimating prothrombin concentration from the clotting time. (Quick, A. J., J. A. M. A., 109: 66, 1937.)

They prepared a dried spinach tablet for use as a reference sample which by their method contained 500 units of K per gram.

There have been various modifications of assay methods developed by different laboratories, notably that of Almquist and associates, Ansbacher and Thayer and associates.

With increasing evidence that vitamin K is concerned with blood clotting because it stimulates production of prothrombin

it was necessary to develop a test for clinical use that would measure prothrombin content of the plasma. The most commonly used test for this purpose is that of Quick. The essential feature of Quick's method is that he eliminates thromboplastin and calcium as variables by adding these substances in excess and thus relates clotting time solely to prothrombin content. We produce Quick's chart in figure 27 by means of which, with his method, clotting time is convertible into per cent of normal prothrombin content.

Referring to figure 20 we see that using his method, the normal clotting time is 12 to 13 seconds. If in a test the clotting time is 30 seconds the chart shows that blood prothrombin content is only 15 per cent of normal concentration.

#### FORMS OF VITAMIN K

Two forms of vitamin K known respectively as  $K_1$  and  $K_2$  have been demonstrated to occur in natural products. One isolated from alfalfa had a smaller molecular weight than the other which was isolated from putrefied fish meal. The product from alfalfa was called vitamin  $K_1$  and that from the fish meal, vitamin  $K_2$ . Both of these compounds were found to be related to a compound isolated from tubercle bacilli by Anderson and Newman. This substance, phthiocol, was demonstrated to be 2-methyl-3-hydroxy-1,4-naphtho-quinone. Vitamin  $K_1$  turned out to be the same compound but with a phytyl group in place of the 3-hydroxy group of phthiocol and vitamin  $K_1$  therefore is 2-methyl-3-phytyl-1,4-naphtho-quinone ( $C_{31}H_{46}O_2$ ). This structure has been confirmed by synthesis. Vitamin  $K_2$  appears to be 2,3-di-substituted 1,4-naphtho quinone.

Because of these discoveries a series of naphthoquinone products were tested for vitamin K activity and it soon became evident that 2-methyl-1,4-naphthoquinone was quite as effective as either of the naturally occurring K vitamins. In fact, it has been suggested by the League of Nations Com-

mittee that the unit for vitamin K activity be taken as one microgram of pure 2-methyl-1,4-naphthoquinone.

At present writing Ansbacher, Fernholz, and Doliver have reported the minimum effective doses of five synthetic products in contrast to natural  $K_1$  given in table 41.

These results would indicate that methyl-naphtho-quinone or methyl-naptho-quinone-hydroquinone is the most active vitamin K compound known and Ansbacher et al. report that it is as effective intravenously in an aqueous medium as it is orally in oil solution. When fed by mouth its potency is greater in water than in oil.

TABLE 41

| MINIMAL EFFECTIVE DOSES OF                        | IN 6 HOURS | IN 18 HOURS |
|---|------------|-------------|
|   | gamma      | gamma       |
| 1. 2-methyl-1,4-naphtho-quinone                   | .5         | .25         |
| 2. Sodium 2-methyl-1,4-naphtho-quinone-hydro-     |            |             |
| quinone diphosphate                               | 10         | 5           |
| 3. Sodium 2-methyl-1,4-naphtho-hydro-quinone      |            |             |
| disulfate   | 25         | 5           |
| 4. Purified phthicol (2-methyl-e-hydroxy-1,4-     |            |             |
| naphtho-quinone)                                  | 2000       | 1000        |
| 5. 2-methyl-1,4-naphthalene-dioxy-di-acetic acid. | 2000       | 1000        |
| 6. Natural K <sub>1</sub>                         | 15         | 1           |

#### SOURCES OF VITAMIN K

Assay methods have not been in operation for sufficient time as yet to permit tabulation of the K values of common food-stuffs. Vitamin K appears to be concentrated in those parts of plants where photo-synthetic activity is present; i.e., in the chlorophyll containing fraction. We know, for example, that it is present in generous quantity in alfalfa, spinach, kale, dried carrot top, chestnut leaves, tomatoes, and oat sprouts. The K<sub>2</sub> found in putrified sardine meal indicates that it is synthesized by certain bacteria and several strains of bacteria, namely, Escherichia coli, B. subtilis and Staph. aureus have been

shown capable of producing it. Since these bacteria are frequently present in the human digestive tract, it is possible for the vitamin to be produced in the digestive tract even if the diet does not supply it.

Recent development of synthetic products has supplied the physician with a variety of preparations for both oral and parenteral use.

#### PHYSIOLOGICAL PROPERTIES

It is not known whether vitamin K enters into the formation of prothrombin as a chemical constituent or merely keeps certain tissues (in the liver, in particular) in a state of activity essential to prothrombin production.

Warner et al. report that the liver is definitely concerned in the manufacture of prothrombin and Smith et al. have shown that when the liver is partially excised or injured by poisons, infections or tumor growth, the level of plasma prothrombin falls.

It is also known that the absorption of vitamin K is favored by the presence of bile salts. Lack of K as a cause of hemorrhage was first noted in human beings in cases of obstructive jaundice.

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## CHAPTER XXII

## VITAMIN K DEFICIENCY

## EXPERIMENTAL VITAMIN K DEFICIENCY

Dam's original experiments, which were devoted to sterol metabolism, simply mentioned the hemorrhage and weakness observed in certain chicks and that orange juice did not correct the disorder. More information was given in a later report (Dam and Schønheyder). Hemorrhages had been noted in 60 to 70 per cent of the chicks reared on the basal The sign appeared after 11 days of depletion, in most cases between the 15th and 20th days. The location of the hemorrhages was determined largely by trauma. Thus the left breast was the common site because injections were made there. Hemorrhage could be induced by pinching. hemorrhages were predominantly subcutaneous and of various sizes. Intramuscular hemorrhages were very common in the legs and often the wings were marked by extravasa-There was no predilection for the knee joint although in a few cases blood was found within the joint. Subperiosteal hematomata were never seen. In a few animals considerable amounts of blood were found in the peritoneal cavity and petechiae were seen at times in the liver. Some chicks were edematous, one had a retrobulbar hematoma.

Brownish areas of a frayed or ulcerated appearance were found in the gizzard. The pigmentation was due to blood. These lesions varied in size from minute spots to areas 1 cm. in diameter. In some cases tarry material was found in the gizzards. In a few chicks patches of hyperplastic epithelium were seen in the cardia. The gizzard lesions consisted

of congestion, hemorrhagic infiltrations and superficial ulcerations. Atrophic changes occurred in the gastric glands, the columnar cells becoming shortened, the gland fundidilated.

McFarlane, Graham and Richardson described their experience with vitamin K deficient chicks as follows. The mortality rate was very high, half of the deaths being due to hemorrhage following the insertion of identification bands into the wings. Bleeding from the small wounds so made continued for from 12 to 24 hours, the feathers being continuously wet with blood. In the remaining chicks large hematomata were found beneath the skin along the femur, particularly the left, the ribs and pectoral muscles. Blood from such animals failed to clot on standing overnight.

Dam, Schønheyder and Lewis had little success in producing vitamin K deficiency in laboratory animals. Hawkins and Brinkhous demonstrated that deficiency could be induced by establishing a biliary fistula and similar results were secured in rats by Greaves and Schmidt. In these animals as in the chick the important lesion was a failure of the blood to clot apparently due to diminished prothrombin. A very satisfactory technique for establishing deficiency in the rat has been devised by Flynn and Warner and consists of placing rats on a diet low in vitamin K from the time of weaning and later ligating the common bile duct. A degree of deficiency can be induced by dietary means alone but never profound enough to depress the prothrombin value greatly. A severe depletion of vitamin K requires deficient intake plus biliary obstruction. Under these circumstances the prothrombin concentration falls slowly during the first 24 hours and thereafter rapidly until after 3 days the values are characteristically 10 to 25 per cent of normal. At the lower value rats invariably bleed unduly from venepuncture or scratches, at 25 per cent they frequently do. Biliary occlusion alone is more effective than simple depletion in establishing a severe prothrombin

deficiency but even after 3 weeks of biliary obstruction the values range between 20 to 40 per cent and thereafter biliary cirrhosis develops and the animals are no longer suitable for most experimental studies. It is interesting to note that under conditions of depletion plus biliary obstruction treatment with vitamin K must be maintained quite constantly. It is difficult to cause a storage of vitamin.

This may be due in considerable measure to the rapid destruction of prothrombin. The studies of Andrus, Lord and Kauer reveal that in hepatectomized dogs the prothrombin level falls within a few hours and may reach a value of only 5 per cent of normal within 14 hours. This is probably due to removal of the site of prothrombin formation and the naturally rapid rate of destruction of the prothrombin of the blood. By measuring the values in blood taken from both sides of the heart these authors found that the values in the systemic circulation were consistently about 10 per cent less than in the pulmonic circulation, the reverse of the concentration of blood platelets. Such a rapid rate of destruction quickly depletes the blood concentration.

### VITAMIN K DEFICIENCY IN MAN

In 1937 Quick suggested, on the basis of the observations of experimental vitamin K deficiency, that cases of obstructive jaundice in man, so commonly complicated by a tendency to bleed, might benefit from treatment with vitamin. The following year Warner, Brinkhous and Smith reported success in such patients and shortly afterward Butt, Snell and Osterberg and Dam and Glavind announced like results. Extensive application of this useful discovery has been made since that time. The methods for determining prothrombin values, all of which are indirect, have usually been either the measurement of the time required to coagulate (Quick's method) or the dilution of plasma which will clot in a specified period (Warner, Brinkhous and Smith). Both methods

have been shown to be satisfactory in identifying cases suitable for treatment with vitamin K. The limitations of Quick's method are chiefly that the level of prothrombin cannot be closely estimated from the time required for clotting although cases with a hemorrhagic tendency regularly show prolongation of the clotting time to some degree. It is not always possible to distinguish between a serious and a minor prothrombin depletion by Quick's method according to Snell, Butt and Osterberg.

The same authors report that in human cases normal clotting time could be reestablished in 1 to 3 days by feeding vitamin K. With this handling the prognosis in jaundiced patients requiring surgical interference has been greatly improved. A general estimate of the mortality rate under such circumstances which can be directly attributed to hemorrhage appears to be about 15 per cent. This can be eliminated by preventive treatment with vitamin K in all but those exceptional patients in whom liver damage is so extensive that prothrombin formation is not adequate despite ample intake of vitamin K. The superiority of vitamin treatment over transfusions has been emphasized by various writers. Treatment should be continued post-operatively because the prothrombin values tend to fall during the post-operative period.

#### HEMORRHAGIC DISEASE OF THE NEW BORN

In addition to instances of prolonged clotting time associated with absence of intestinal bile vitamin K has been found to be most useful in controlling and preventing the hemorrhagic diathesis in new born infants. The low prothrombin values in babies was pointed out by Brinkhous, Smith and Warner and Waddell and Guerry early made successful use of vitamin K in such cases. The usual course of events in infants is that the birth values are adequate but that during the first few days of life they fall precipitately

and then slowly recover spontaneously. The explanation of this phenomenon of the new born is not yet clear. Vitamin K can be formed through bacterial action and it was thought that this might explain the behavior of infants, that the birth supply was rapidly depleted until a bacterial flora was established which synthesized sufficient vitamin K. Quick and Grossman point out that this would explain the observation that among the babies of the well-to-do, born in a hospital where sterile care is given the incidence of hemorrhagic disease has been double that among infants cared for through free maternity clinics. The high prothrombin values in women at term does not support the view that the deficiency is secondary

to depletion of the mother.

The explanation proposed by Tocantins is that the liver is at fault. Tocantins observed an infant who became intensely jaundiced and vomited blood and food on the second day of life. This state persisted until the 11th day of life and was associated with an icteric index of 150 units and a plasma prothrombin value of 1 per cent (Quick's method). bleeding time was several hours. On the 11th day the jaundice began to fade and a small bile-tinged stool was passed. prothrombin rose abruptly to 113 per cent of normal and the serum bilirubin dropped. Vomiting persisted however and the infant died. At necropsy the duodenum was found completely stenosed by a membrane proximal to the Ampulla of Vater. Thus food could not enter the jejunum or be mixed with bile and the natural bacterial contamination of the bowel content was also interfered with. Yet the prothrombin spontaneously increased concomitantly with the fall in plasma bilirubin. Tocantins considered the icterus to be an exaggeration of the "physiologic" icterus of the new born. hypoprothrombinemia might therefore be considered exaggeration of the "normal" behavior as well. Therefore they may have a common cause, especially since they are regularly related in time. The livers of new born infants are functionally and structurally immature. Under the added stress of excretion of excess bilirubin it is unable to form prothrombin at a proper rate.

Hypoprothrombinemia may be corrected in the infant by feeding vitamin K or may be prevented by administering

vitamin to the mother before delivery.

## NUTRITIONAL DEFICIENCY OF VITAMIN K IN MAN

Thus the common forms of hemorrhage which can be relieved by vitamin K are secondary. This has been a remarkable feature of the development of our knowledge of this vitamin and one which distinguishes it from all others in which inadequate diets are at fault. But presumably strictly dietary forms of vitamin K deficiency do occur although less commonly. Thus Kark and Lozner describe 4 individuals who had lived for considerable periods on restricted diets and in whom the blood clotting time was increased. One likewise had pellagra and the others were scorbutic. There was no jaundice and all four responded to the oral administration of vitamin K without bile salts. Stewart and Rourke have reported cases of delayed blood clotting without jaundice or hepatic disease and Snell, Butt and Osterberg have found certain patients with ulcerative colitis and other lesions of the colon to have a hemorrhagic diathesis responsive to vitamin K. Mackie investigated 277 patients and found 57 to have delayed clotting time although there was no evidence of jaundice or hepatic disease. The diagnoses in these patients were as shown in table 42.

In several cases severe hemorrhage occurred which seemed related to the prolonged clotting time. Treatment with vitamin K alone was usually successful.

# MINERAL OIL AND VITAMIN K

Vitamin K deficiency due to faulty absorption has been demonstrated experimentally by Elliott, Isaacs and Ivy who noted that rats raised on diets containing 20 per cent of mineral oil developed a hemorrhagic tendency and prolonged clotting time. Gray and Ivy had suggested that vitamin D was effective in the control of hemorrhage in jaundiced patients. Therefore Elliott and his associates tried parenteral D as well as K in their rats. Vitamin D seemed to shorten the clotting time to a moderate degree. Vitamin K was extremely effec-

TABLE 42

| DIAGNOSIS .                   | NUMBER OF CASES |  |  |
|-------------------------------|-----------------|--|--|
| Chronic ulcerative colitis    | 28              |  |  |
| Peptic ulcer                  | 9               |  |  |
| Regional enteritis            | 3               |  |  |
| Lobar pneumonia               | 3               |  |  |
| Gastritis                     | 1               |  |  |
| Gastric carcinoma             | 1               |  |  |
| Postpartum hemorrhage         | 1               |  |  |
| Lung abscess                  | 1               |  |  |
| Chronic hemorrhagic diathesis | 1               |  |  |
| Cardiac insufficiency         | 1               |  |  |
| Pernicious anemia             | 1               |  |  |
| Retroversion of the uterus    | 1               |  |  |
| Hypothyroidism                | 1               |  |  |
| Dietary deficiency            | 1               |  |  |
| Sprue                         | 1               |  |  |
| Banti's syndrome              | 1               |  |  |

tive. It would therefore seem that in the rat a sustained high mineral oil diet is capable of inducing a vitamin K deficiency.

# THE ADMINISTRATION OF 2-METHYL-NAPHTHOQUINONE

The control of prothrombin deficiency by vitamin K is best achieved through the use of 2-methyl-naphthoquinone which may be given intravenously or intramuscularly as well as orally. The dose is 1 to 2 mgm. If given orally in patients with biliary obstruction bile salts should be administered simultaneously. Although the action of vitamin K is extremely rapid it is evident that the prevention of hemorrhage

is superior to treatment of hemorrhage once it has occurred. Suspected cases should be examined by means of one of the tests of prothrombin activity described in Part II of this book. If the clotting time is delayed vitamin K should be given to prevent hemorrhage and the administration should be continued for several days.

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# CHAPTER XXIII

# THE VITAMINS AND INFECTIOUS DISEASES

One of the oldest of medical concepts is that nutrition is vital to resistance to disease. To transform this generality into specific phenomena and to associate them with specific nutritional factors has been the ambition of many an experimenter. Resistance to infection being an extremely complicated affair, matched only by the complexities of nutrition, it is not surprising that progress has been slow. A great deal has been accomplished but not much of a thoroughly satisfactory character. Many nutritionists have proved to be very uncritical microbiologists and many microbiologists completely indifferent to the diets fed their animals. A great deal of arbitrary selection has therefore been necessary in composing the following résumé.

### VITAMIN C

In the laboratory infection is most frequently seen in animals on a diet deficient in ascorbic acid. Scurvy and infectious diseases go hand in hand. It is not surprising, therefore, that the most extensive literature exists regarding the part this factor plays in maintaining resistance to infections. Indeed the first reference to experimental scurvy was made in connection with an infectious disease. In 1895 Theobald Smith, while studying swine erysepelas in guinea pigs, observed the death of an injected animal in which both hind legs showed extensive hemorrhages. Smith wrote:

To explain the death of this guinea pig it will be necessary to record some facts which have been observed for some years in this laboratory. When guinea pigs are fed with cereals (bran and oats mixed), without any grass

clover, or succulent vegetables, such as cabbage, a peculiar disease, chiefly recognizable by subcutaneous extravasation of blood, carries them off in from four to eight weeks. The death of No. 254 was undoubtedly due to the absence of such food, as the attendant had neglected to provide it after the disappearance of grass in the fall of the year. Furthermore, No. 255 was weakened by the restricted diet and succumbed to an inoculation which otherwise might have had no visible effect. (Ital. ours.)

Similar observations are commonplace. A recent report serves as a good example of what a scurvy producing diet will do to rhesus monkeys. Sabin kept 25 animals on such a diet during his study of the effect of ascorbic acid on experimental poliomyelitis. Thirteen died between the 9th and 18th days while 21 animals on a full diet remained well. The causes of death were: lobar pneumonia, 5; hemorrhagic colitis, 3; erysipelas-like infection, 1; cause not established 4.

Many inquiries have been made of the influence of ascorbic acid on the course of tuberculosis. This has been due, no doubt, to the realization of the large part diet plays in the control of clinical tuberculosis, a phenomenon which has never been satisfactorily explained. Tuberculosis and scurvy have a common history. If one investigates the epidemics of scurvy one is constantly reminded of the simultaneous frequency of tuberculosis. Aschoff and Koch's large series of necropsies of scorbutics revealed that tuberculosis was the common cause of death. Of those due to other diseases most

Scurvy modifies the anatomical responses to tuberculosis in at least two ways, by predisposing to hemorrhage and by preventing the evolution of a proper fibrous tissue scar. Robert Koch said that scurvy was a serious hazard to tuberculous individuals because of the tendency to hemorrhage. The point of inoculation of tubercle bacilli frequently becomes hemorrhagic and even the lesions in the spleen and other organs are surrounded by extravasated blood.

were the result of other infectious diseases.

Höjer extensively studied the histopathology of experimental

tuberculosis as it is influenced by diet. Two of his illustrations are particularly illuminating. They contrast the collagen poor, irregular scar about a tuberculous focus in an animal moderately depleted of vitamin C and the solid, compact scar in a well fed pig. This appears to be a thoroughly reliable observation which harmonizes with what has already been said of the influence of ascorbic acid on the formation of collagen. The same mechanism probably explains those cases seen by Aschoff and Koch in which old tuberculous lesions were activated and an acute, exudative inflammation spread from them. Mazoué's work is confirmatory. Wolbach has written: "In diseases, notably the infectious granulomata, where fibrosis of lesions is the important element in arrestment of progress, avoidance of C deficiency is of supreme importance." It may be necessary to broaden this statement. Wachsmuth and Heinrich reported a case of scarlet fever complicated by starvation. Four months later bone pain led to an exploratory operation. A sequestrum, infected with streptococci, was found. In commenting on this case Takahashi ascribed this metastasis of the infection to the nutritional condition (probably scurvy) and said that in his own experiments scurvy had produced foci of low resistance so that pathogenic microörganisms injected intravenously induced disseminated lesions in all scorbutic guinea pigs but in less than half of the controls.

The sensitivity to tuberculin has been investigated. Recent studies by Birkhaug illustrate the general outcome of such studies. Birkhaug tested two groups of 12 guinea pigs each for sensitivity to tuberculin on the 24th, 51st and 62nd days following injection of a uniform dose of bovine tubercle bacilli. Both groups were fed an "adequate" diet but one received a supplement of 10 mgm. ascorbic acid daily. Chemical tests of urine and adrenal tissue revealed that the control group became slightly depleted of vitamin, the experimental animals remained saturated. They were likewise less sensitive to

tuberculin. Dissection and histologic studies confirmed Höjer' results. The ascorbic acid poor pigs showed more caseous lesions, less collagenous scar and more dissemination of the infection. Heise, Martin and Schwartz have conducted similar studies in human cases. The administration of ascorbic acid supplements was shown to reduce the skin sensitivity to tuberculin. The 24 hour erythema readings in the controls averaged 351 sq. mm. as compared to 76 sq. mm. in the patients given ascorbic acid. In another of a series of articles on the subject Heise and Martin reported a failure to demonstrate any benefits in tuberculosis from massive doses of ascorbic acid.

Comprehensive experiments and clinical investigations have been reported by Steinbach and Steinbach and Klein. In a discussion of the phenomena in experimental (guinea pig) scurvy they state that ascorbic acid treatment over a considerable period leads to a heightened resistance to tuberculin. They were able to establish conditions under which the treated guinea pigs survived doses of tuberculin which regularly killed control animals. The adrenal glands were examined for ascorbic acid (silver nitrate test) and it was discovered that the cortex was depleted of vitamin in those animals which had been killed by tuberculin.

We have ourselves observed among pigs injected with routine sputum concentrates and urine that animals on an inadequate diet develop more extensive lesions and are more susceptible to tuberculin shocking than pigs adequately fed.

Clinicians have generally reported benefits from vitamin C rich diets but in most of these reports the differences have not been as striking as those in experimental tuberculosis or, it seems proper to suggest, as they would be under circumstances in which the basal diet was deficient. Clinical studies are in agreement in another respect. Patients suffering from tuberculosis are almost uniformly found to have abnormally low blood values of ascorbic acid, to be partially depleted.

The same is true for most infectious diseases. On the other hand capillary fragility, another sign of scurvy, is seldom present in clinical tuberculosis. An exception appears to be the patients with hemoptysis. Borsalino investigated such cases. Capillary fragility was frequently present in cases with hemoptysis and was amenable to treatment with ascorbic acid. Hemoptysis ceased as the fragility disappeared and recurred when treatment was discontinued.

In the first edition of this book we discussed the studies of Rinehart who observed changes in experimental scurvy which were suggestive of those in rheumatic fever. Rheumatic fever is a disease of the poor and this and other characteristics of it suggested a nutritional relationship and led Rinehart to consider mild forms of scurvy worthy of further study. Many investigators have explored the problem since then. hart's observations have been confirmed but his interpretation has been the subject of much disagreement. Disagreement has centered on two points. The lesions have not appeared to all pathologists to be characteristic of rheumatic fever and the theory that subclinical scurvy has etiological significance has been denied on the basis of clinical trials in which even considerable amounts of the vitamin have failed to prevent recurrences. The blood of rheumatic fever subjects is usually depleted of ascorbic acid, as in the case of tuberculosis, but that is true of infectious diseases as a class. However, not many infectious diseases seem to influence the blood levels as greatly as rheumatic fever does. Rinehart reports that even cases of rheumatoid arthritis, in 93 per cent of his cases, had blood values below 0.5 mgm. per cent. Rheumatic fever patients usually have capillary fragility as well as low blood levels of vitamin.

Several observations have been made in the course of these studies which are of considerable interest. Taylor believes that scurvy produces a carditis without superimposed infection; that if allowed to persist it reaches a stage of irreversibility as far as ascorbic acid influence goes. Valvulitis, myocarditis, pericarditis and arthritis all occurred in his animals and the carditis was capable of causing congestive heart failure. The lesions frequently contained microörganisms although none were injected indicating that a latent infection had been activated by the scurvy. In Rinehart and Schultz' experiments infection was induced by injecting streptococci. The present status of this problem would seem to be that definite evidence of the part played by ascorbic acid in the etiology of rheumatic fever is lacking but that the synergistic effect of mild scurvy and infection has been confirmed in critical and extensive experiments.

The most recent addition to these studies has been made by Schultz and Schultz and Rose. Schultz sought to explain the findings of Rinehart on the basis of increased metabolism. Both thyroxin and insulin were found capable of producing the type of carditis associated with scurvy. Whether this will be the final explanation of the influence of vitamin C or not remains to be seen. Evidently the action is not a specific one.

The effect of vitamin C on toxins has been the subject of many reports. Harde was a pioneer in this field. She felt a considerable similarity existed between the lesions of scurvy (in the adrenal) and those due to the injection of diphtheria toxin and that animals, like the mouse, which synthesize vitamin C were resistant to certain toxins because of this ability to synthesize ascorbic acid while guinea pigs which are susceptible to the toxins are susceptible because they are dependent on dietary vitamin. These observations led to experiments which seemed to demonstrate that ascorbic acid was capable of both an in vitro and in vivo inactivation of diphtheria toxin. King and Menten and Jungeblut and Zwemer reported similar results and associated the effect of the toxin on the adrenal with its ascorbic acid content. Pakter and Schick have reviewed these and other experiments and ascribe the effect to the reducing property of ascorbic acid and

in certain experiments to the pH of the mixtures used. They failed to correlate vitamin C with the Schick reaction in children. The experimental evidence is quite contrary and seems to demonstrate a direct effect. Torrance's experiments, for example, clearly show an inverse correlation between adrenal hemorrhage and vitamin C content and that additional vitamin C reduces both the hemorrhagic response to diphtheria and mortality. Torrance speaks of the adrenal changes as a "localized scurvy." Torrance found meningococcus filtrates capable of the same effect on the adrenals and their vitamin content. Frequent clinical reports speak of the benefits to be derived from treating diphtheria with ascorbic acid and antitoxin. Kumagai wrote that 400 to 600 mgm. daily reduced the death rate among cases of severe, necrotizing diphtheria. The subject of ascorbic acid and toxins must be considered as open.

Jungeblut extended his observations of the effect of vitamin C on diphtheria toxin by studies of the action on the virus of poliomyelitis and on the experimental disease. The virus was rapidly inactivated in vitro. Animal experiments seemed to show that doses of ascorbic acid at a definite level gave protection against the disease. However Sabin was unable to confirm this. In a discussion of the relationships between ascorbic acid and poliomyelitis Heaslip reported observations made in Australia. He had noted the frequent reference in the literature to the common susceptibility to diphtheria and poliomyelitis. In 1917 Zingher found that a disporportionately high percentage of poliomyelitis cases in New York City occurred among Schick positive children. This was also true of the Australian cases. Heaslip also studied the urinary excretion of vitamin C and showed that a further characteristic of such children was a low excretion of ascorbic acid, that is a form of depletion.

These and similar studies have brought sharply to our attention the significance of different levels of vitamin C nutri-

tion. Dietary habits are doubtless of the greatest importance in determining this characteristic but individual factors are also important. It seems justified to ask whether these are all but expressions of an underlying constitutional character rather than the reflection of eating habits.

In the matter of vitamin C metabolism during febrile diseases it has been learned that most, if not all, febrile diseases do lower the vitamin C levels in blood and tissues. Frequently this depletion deepens to a stage of significance to the recovery of the patient. The action is not understood. Daum, Boyd and Paul and Zook and Sharpless have shown that artificial hyperpyrexia causes a fall in blood vitamin. Possibly fever alone is responsible. The intake of ascorbic necessary to maintain "normal" level in the blood plasma is often quite large during infectious diseases. Faulkner and Taylor describe a case of tuberculosis in which 200 mgm. per day were needed. We have followed patients who required still more.

One further aspect of this problem seems important. A study of the symptoms of many cases of prolonged infectious diseases reveals many lesions which may be due in part or entirely to secondary vitamin deficiency. The Zenker's degeneration of the abdominal muscles and excessive bleeding in typhoid fever may be better explained by a superimposed scurvy than by the typhoid itself. By high calorie diets Coleman radically improved the prognosis and shortened the duration of typhoid fever. It is reasonable to believe that further improvement could now be made by providing an adequate vitamin intake.

A comprehensive study of the effect of vitamins on one factor in resistance to disease, specific antibody production, has been made by Jusatz. Rabbits were used. They were fed autoclaved diets which caused a form of ill health. To this were added various vitamins and the various groups were then immunized to horse serum. The criterion used was the formation of precipitins. Oral administration of vitamins

A, B complex, D and C was without effect but the subcutaneous injection of large amounts of ascorbic acid trebled the titer of the serum. Jusatz also found that the ultimate titer of the serum was increased by intravenously administered ascorbic acid, injected immediately preceding the injection of antigen.

#### VITAMIN A

Some years ago vitamin A was frequently spoken of as the "anti-infectious" vitamin. This was due to early studies of rat avitaminosis. McCarrison and Mellanby both emphasized the frequent association of inflammatory lesions. Mellanby, for example, reported that among 92 rats on an A deficient diet 44 per cent had urinary tract infections, 20 per cent had otitis media, 21 per cent enteritis, 9 per cent pneumonia and 95 per cent abscesses in the floor of the mouth. For a time indeed it was believed that infection preceded the epithelial metaplasia and produced it.

Later work disproved this view. It is now generally conceded that the metaplasia is the primary effect of deficiency, that it is of such a nature that the impenetrability of the epithelium is destroyed and that microörganisms are thus enabled to invade the epithelial surfaces which are affected. Vitamin A is "anti-infectious in the sense that it preserves the normal cellular barriers against infection. The effect may not be entirely structural. Thus Sullivan and Manville found changes in the lysozyme production of the intestinal epithelium. Lysozyme is the name given the bactericidal factor discovered by Fleming in many body fluids. Findlay, in 1925, and Anderson, in 1932, had shown that its production is diminished in vitamin A deficiency. In 1937 Prickett, Miller and McDonald reported it was increased. and Manville were able to explain this discrepancy. found that while the content of the bowel wall was increased the secreted lysozyme was greatly reduced in amount.

The indispensability of vitamin A to epithelial structures suggest that these phenomena may be characteristics of this deficiency alone. Possibly the same mechanism is responsible for the many claims that vitamin A is preventive of common colds. Cod liver oil and concentrates of cod liver oil have enjoyed a widespread popularity as cold preventives. Beard reported that the severity of colds was reduced among 36 students who had taken cod liver oil steadily throughout a year. Holmes and his associates, working with an industrial group, reported a reduced incidence of infection and 40 per cent less loss of time among employees given cod liver oil. Shibley and Spies, on the basis of an extensive experiment, concluded that vitamin A had no effect on the incidence or severity of colds although there was suggestive evidence that the duration of the illness was shortened. Gardner and Gardner found that the severity and duration of colds was reduced in a group given supplements but no reduction in the average number of infections.

The common cold is a complex infection in the sense that it is inaugurated by a virus and that the prolongation of the illness and the later symptoms are due to secondary bacterial infection. This may explain the results just summarized. Thus the respiratory epithelium could conceivably be no more resistant to invasion by the primary pathogen, the virus, and yet be more resistant to the secondary, bacterial infection. Under such circumstances the incidence of colds would not be reduced but their duration and severity would be.

At the present this is purely speculative since there is no agreement that the course of the common cold is definitely influenced by vitamin A supplements. The problem cannot be solved until much more is known of intake and requirements. Certain observers have had favorable results and others unfavorable because the nutritional status of their patients was quite different. Thus Hess and Barenberg found that vitamin A did not prevent or reduce the incidence

of the common infectious diseases among the infants in their institution but since dietary problems had been earnestly studied there for many years we may assume that the basal diet, to which supplements were added, was fully adequate to begin with.

Some experimenters have attempted to measure the relationship of vitamin A and resistance more accurately. Clausen's experiments require attention on this account. He correlated dietary supply of vitamin and plasma carotene content with resistance to infection. Infectious diseases were more common among infants 6 to 24 months old who had not received cod liver oil or vegetables and whose plasma carotene was low. Scarlet fever was less severe in children whose plasma carotene was of a high value at the beginning of the illness. Plasma carotene fell during infectious diseases, especially during the more prolonged illnesses. This was due in large part to the reduced food intake. Clausen obtained certain anomalous results which cannot be explained at present but his experiments point the way to better study of such problems.

Various attempts have been made to determine whether vitamin A has value after the infectious agent has passed the epithelial barrier. Boynton and Bradford demonstrated that A deficient rats were less resistant to certain virulent microörganisms injected intraperitoneally. Smith and Hendrick reported that deficiency reduced the resistance to tuberculosis. Lassen extensively studied the course of salmonella infections in deficient rats. The deficiency predisposed to generalization of the infection. McClung and Winters had similar results. Repeated passage through deficient animals did not increase virulence, however. Lassen demonstrated some inferiority in the formation of antibodies. His monograph and that of Robertson may be recommended

for a review of the literature.

During vitamin A deficiency the Kupffer cells become swol-

len and degenerate. It is quite possible that a part of the effect of vitamin A deficiency on infectious diseases is due to this involvement of the reticulo-endothelial system.

In most experiments in which an increased susceptibility has been clearly shown the degree of deficiency has been considerable. Thus Lassen found the stage of diminished resistance present only when xerophthalmia was evident. Comparable degrees of deficiency are not common among our sick.

The stigmata of vitamin A deficiency are relatively permanent as has been mentioned in an earlier chapter. Sherman and MacLeod found that deficiency during youth conditioned the resistance throughout life. Thus in rats "there appeared in early adult life an increased susceptibility to infection, and particularly a tendency to break down with lung disease at an age corresponding to that at which pulmonary tuberculosis so often develops in young men and women."

### VITAMIN B

In the early studies of vitamin B and infectious diseases the B complex was widely used and it is impossible to assign the results to particular components of the complex. Perla's review may be consulted for work published prior to 1938. The most extensive work was reported by Kuczynski who used various diets of natural foodstuffs and tested the resistance of mice to intracerebrally injected yellow fever virus. B<sub>1</sub> deficiency was most effective in reducing resistance.

Leprosy has frequently been used as an experimental disease because clinicians familiar with it have observed the importance of diet. Thus Lamb demonstrated that diets low in B and calcium reduced the time required for the evolution of leprosy nodules in rats and caused more extensive lesions. He extended his studies over several generations of animals on a slightly deficient diet and discovered that the 3d and 4th generations showed an increased susceptibility. These studies

have been extended by the work of Badger and Masunaga who confirmed Lamb's results on incubation and dissemination. Supplements of thiamin chloride completely corrected the susceptibility. The influence of calcium, also noted by Lamb, proved to be indirect. The calcium depletion caused a deficiency in thiamin. Despite calcium depletion normal resistance to leprosy could be maintained by large amounts of vitamin B<sub>1</sub>. A representative measure of resistance was the time at which palpable lepromata appeared.

TABLE 43

| EXPERI-<br>MENT<br>NUMBER | DIET                         | NUMBER<br>OF<br>RATS | PER CENT OF RATS HAVING PALPABLE LEPROMATA AT VARIOUS WEEKS |        |            |             |            |              |
|---------------------------|------------------------------|----------------------|---|--------|------------|-------------|------------|--------------|
|                           |                              |                      | First   | Second | Third      | Fourth      | Fifth      | Sixth        |
| II                        | Thiamin deficient<br>Control | 25<br>25             | 0   | 40     | 68<br>24   | 100<br>56   | 96         | 96           |
| VIII                      | Thiamin deficient<br>Control | 24<br>24             | 0   | 0      | 8.3<br>4.1 | 29.1<br>8.3 | 50<br>33.3 | 87.5<br>79.1 |

Many epidemics of typhus fever have been associated with periods of famine and a relationship between diet and typhus has been sought. Zinsser, Ruiz Castaneda and Seastone found that scurvy, in the guinea pig, increased the susceptibility to typhus. They inoculated their animals after symptoms of scurvy appeared. In such animals the rickettsiae were more widely distributed and more numerous than in adequately fed animals. Vitamin C deficient diets increased the susceptibility of rats also but not to the same extent.

More recent work (Pinkerton and Bessey) shows that riboflavin deficiency is even more effective in reducing the resistance of rats to typhus fever. This study is of great interest because it represents the successful application of a known characteristic of a deficiency disease. Rickettsiae are intracellular organisms and as such are much affected by the

health of their host cells. Cellular oxidation is reduced in riboflavin deficiency. Therefore conditions affecting the growth of rickettsiae might be presumed to occur during deficiency. This proved to be the case. The organisms were widely disseminated throughout the organs and were present in tremendous numbers.

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# PART II

METHODS OF STUDYING AVITAMINOSES.
VITAMIN VALUES OF FOODS



# APPENDIX A

# LABORATORY TESTS USEFUL IN THE DIAGNOSIS AND STUDY OF DEFICIENCY DISEASE

BLOOD PLASMA CAROTENE AND VITAMIN A

We have used the modification of van Eekelen's method described by Menken. A 20 cc. sample of citrated blood is centrifuged and the plasma removed. Ten volumes of 95 per cent alcohol are then added and the alcohol fraction centrifuged again. The alcoholic fraction is then well shaken with petroleum ether and the extract evaporated to 1 cc. and the carotene content estimated by comparing it with a known solution of carotene or a potassium bichromate equivalent. The sample is then evaporated to 0.2 cc. and 1 cc. of a saturated solution of antimony trichloride in chloroform is added as well as one drop of acetic anhydride. The blue color produced is immediately measured in a Lovibond tintometer or compared with solutions of copper sulphate in ammonia solution.

Such standards are made as follows. Two grams copper sulphate are dissolved in 50 cc. water and 10 cc. of a 20 per cent solution of ammonia added. The solution is diluted to a volume of 100 cc. One cubic centimeter of this solution plus 5.5 cc. water is equivalent to 5 Lovibond blue units. Two cubic centimeters of this solution plus 1 cc. 2 per cent ammonium hydroxide is equivalent to 4 units, 1 cc. plus 1 cc. ammonium hydroxide is equivalent to 3 units, 1 cc. plus 2 cc. ammonium hydroxide is equivalent to 2 units and 1 cc. plus 5 cc. ammonium hydroxide solution is equivalent to one Lovibond blue unit.

It has been our practice to extract the alcohol fraction three times with 50 cc. petroleum ether, to evaporate the extract to approximately 5 cc. and then redissolve in a small amount of petroleum ether and allow the latter to remain over night in a vacuum desiccator. The acetic anhydride is unnecessary under these conditions since it serves only to prevent the formation of a cloudy solution which results if a small amount of moisture is present.

Menken found the vitamin A values of blood plasma to be between 0 and 8.4 Lovibond units by this method, results with which we are in general accord.<sup>1</sup>

For more accurate determinations use samples of 5 cc. plasma, treat with 0.5 cc. 60 per cent alcoholic potassium hydroxide and reflux for 5 minutes in a boiling water bath. The cooled mixture is then treated with half its volume of 95 per cent alcohol and extracted three times with petroleum ether to give about 35 cc. of extract. This is washed with water, dried with anhydrous sodium sulfate, filtered and transferred to a 100 cc. flask where it is evaporated with a stream of nitrogen. The residue is taken up in 1 cc. petroleum ether, dried with a small amount of sodium sulfate and diluted to 2 cc. The carotene can then be determined in a photometer or by comparison with a 0.02 per cent potassium dichromate solution.

Vitamin A is determined by evaporating the sample to dryness and dissolving the residue in 0.25 cc. of chloroform. The saturated antimony trichloride (in chloroform) solution is added and the blue color either matched against a solution of copper sulfate or read in a photometer. Both colors follow Beer's law.

Photometric determinations of the antimony trichloride reaction require complete protection against clouding from moisture. In our experience the best means of accomplishing this is by using a closed system which contains the reagent

<sup>&</sup>lt;sup>1</sup> Menken, J. G., Deutsche med. Wchnschr., 58: 1484, 1932.

in a separate compartment which may be emptied into the chloroform extract of the plasma by means of a stopcock. By this means the photometer may be adjusted and with little delay the solutions mixed without exposure to moisture.<sup>2</sup>

# SEPARATION OF CAROTENE FROM OTHER PIGMENTS

Carotene may be readily separated from other plant pigments by filtration through a column of finely divided soda ash, washing with petroleum ether until it comes through clear. A chromatograph of the various pigments forms in the column, the carotene is recovered from the filtrate.<sup>3</sup>

# THE MEASUREMENT OF NIGHT BLINDNESS

Many devices have been used in determining the presence and degree of night blindness. A simple test can be made with a pocket watch with luminous dial by testing the patients range of vision before and after a period in complete darkness. Birch-Hirschfeld improvised a simple apparatus in which five holes in a small field are unequally illuminated by means of a Goldberg wedge, a glass plate of increasing optical density from one end to the other. The target is mounted in a lightproof box which contains a small electric light and an iris diaphragm by means of which the intensity of the illumination may be regulated. The procedure is to determine the intensity of light required by the patient to enable him to see three of the holes in the target immediately after coming from a brightly illuminated room and again after ten minutes rest in darkness.

Birch-Hirschfeld's apparatus is manufactured by Carl Zeiss, Inc. A simple substitute can be made using a microscope lamp in which the target is mounted, equipping the lamp with a fifteen watt bulb and modifying the intensity by connecting a variable resistance in series. A resistance coil with

<sup>&</sup>lt;sup>2</sup> Pett, L. B., and LePage, G. A., J. Biol. Chem., 132: 585, 1940.

<sup>&</sup>lt;sup>3</sup> Kernohan, G., Science, 90: 623, 1939.

sliding contact and of 6000 ohms and 0.2 ampere capacity is suitable. The intensity of the light at various positions of the sliding contact may be measured by a photometer and the intensities thereafter calculated from the resistance.

A still simpler and less expensive apparatus may be made from a toy movie projector in which the light source is a small lamp operated by flashlight batteries. In the film track is inserted a copy of the target we have illustrated, printed on a photographic film. A micro ammeter and 20 ohm variable resistance are connected in series and the image is projected on a suitable screen. The advantages of this device are that the image is much more easily seen than the small target in the Birch-Hirschfeld type of photometer, construction is simpler and the ammeter readings approximate the intensity of the light. The machine is easily moved about. The total expense is less than five dollars.

The method of testing should be practiced and the results standardized on normal individuals. The distance from the target and all other conditions must be uniform. Cases showing relatively poor vision after ten minutes in the dark can be tested therapeutically by feeding cod liver oil for several weeks after which dietary cases of hemeralopia should show distinct improvement.

Frandsen used an illuminated wall chart in which the test letters were of various intensities. The more elaborate instruments now on the market need not be described here since the manufacturers can supply information. Pett's instrument is simple and could be readily built with the aid of a tinsmith and an electrician.<sup>4</sup>

#### BLOOD PYRUVIC ACID

Approximately 5 cc. of blood is collected in a tube containing 25 mgm. iodoacetic acid (using a 50 per cent solution

<sup>&</sup>lt;sup>4</sup> Frandsen, H., Acta Opthal., suppl. 4, 1935. Pett, L. B., J. Lab. & Clin. Med., 25: 149, 1939.



PLATE XL. A simple photometer operated by batteries. The ammeter, switch and rheostat are shown. The projection lens protrudes from the upper left hand corner. Behind it is a small strip of film resembling the target shown in the insert. The light is gradually increased in intensity until the subject can recognize the middle circle. The test is then repeated after ten minutes rest in a dark room. After this interval individuals depleted of vitamin A show little or no improvement in their ability to see the middle circle despite the opportunity for regeneration of their visual purple.



adjusted to pH 7.8 with sodium hydroxide) and 20 mgm. potassium oxylate. The blood is then transferred, drop by drop, into a flask containing 12 cc. of 10 per cent trichloracetic acid, shaking continuously. After standing for 30 minutes it is filtered and 3 cc. of the filtrate are transferred to a test tube containing 1 cc. of a 0.1 per cent 2,4-dinitrophenylhydrazine solution in 2 N hydrochloric acid. After standing for 10 minutes the mixture is extracted with ethyl acetate. The extraction is facilitated by bubbling nitrogen through the mixture. The bottom layer is repeatedly extracted with 2 cc. amounts of ethyl acetate until the bottom layer is colorless. The combined extracts are mixed with 2 cc. sodium carbonate for five minutes using a stream of nitrogen and the sodium carbonate extract removed. Two more extractions are made similarly and the combined sodium carbonate fractions are extracted with 1 cc. ethyl acetate and then transferred to the cup of the colorimeter. To the extract are added 4 cc. of 2 N sodium hydroxide. The mixture is shaken and allowed to stand for 10 minutes. The color is then evaluated in the colorimeter using Filter 520. A standard curve must be prepared for each instrument. Normal values lie between 0.77 and 1.16 mgm. per cent. Values above 1.30 mgm. per cent are considered abnormal.5

### URINE NICOTINIC ACID

Two cubic centimeters of the urine is placed in a test tube and 2.5 cc. of a 2 per cent potassium hydrogen phosphate solution added, the mixture then being heated for 5 minutes in a water bath (75 to 80°) after which 0.5 cc. freshly prepared cyanogen bromide solution is added. Heating is then continued for 5 minutes, the tube cooled to room temperature and 5 cc. of a saturated fresh aqueous solution of metol added. Color develops after 30 to 35 minutes (in the dark). The values are conveniently read in a photometer making a cor-

<sup>&</sup>lt;sup>5</sup> Bueding, E., and Wortis, H., J. Biol. Chem., 133: 585, 1940.

rection for the reagents by means of a blank. The color is stable for 6 hours.

Cyanogen bromide solution is prepared by slowly adding 10 per cent potassium cyanide to saturated bromine water at room temperature to the point of decolorization. Metol (Agfa) solutions must be freshly prepared also.

The excretion of normal individuals was found to lie between 3.4 to 10.2 mgm. per day. The values in individuals varies greatly from day to day. Nicotinic acid is absent or present in very small amounts during pellagra.

#### PORPHYRINURIA

As pointed out in the text the presence of reddish pigments in the urine is a characteristic of pellagrins. These may easily be demonstrated by the following method.

Place 10 cc. of urine in a separatory funnel, acidify with glacial acetic acid to pH 4.0 (approximately). About 0.2 cc. of acid is required. Add 15 to 20 cc. ether and shake well. The ether fraction is then washed twice with water and 3 cc. of 25 per cent hydrochloric acid are added. The acid ether mixture is well shaken and allowed to separate. The acid fraction contains pigments ranging from pink to purple in pellagrins. These disappear after treatment.

### ASCORBIC ACID DETERMINATIONS

The simplest procedure for estimating the ascorbic acid content of nearly colorless fluids is visual titration with 2.6 dichlorophenolindophenol. The dye is readily purchased. It should be prepared in such a concentration that 1 cc. is equivalent to 0.02 mgm. ascorbic acid. The dye solution will keep for several weeks and the rate of deterioration is

<sup>&</sup>lt;sup>6</sup> Bandier, E., and Hald, J., Biochem. J., 33: 264 and 1130, 1939. Rosenblum, L. A., and Jolliffe, N., J. Biol. Chem., 134: 137, 1940.

<sup>&</sup>lt;sup>7</sup> Spies, T. D., Sasaki, Y., and Gross, E. S., South. Med. J., 31: 483, 1938.

constant throughout that period so that an arbitrary correction can be made.

(Ascorbic acid can be titrated with iodine using starch as an indicator. Ascorbic acid (milligrams)/iodine (milligrams) = 160/231. An iodine solution approximately 0.006-7 N is suitable.)

Titration of the ascorbic acid content of urine can conveniently be performed in a porcelain dish in which 10 to 20 cc. of urine is acidified (pH 2.5 to 3) with 5 cc. trichloracetic or acetic acid. Five drops of glacial acetic acid per 10 cc. of urine is satisfactory. The dye is run in quickly. The end point is a faint but permanent pink color (lasting at least 30 seconds). Other substances which reduce the indicator do so more slowly than ascorbic acid.

Urine samples should be collected into bottles which contain acid and which are stored in the cold. The best arrangement is to examine specimens promptly. There are no advantages in estimating the 24 hours excretion in clinical practice and many disadvantages.

The ascorbic acid content is readily calculated since the equivalent of each cubic centimeter of indicator is known.

A rough estimation of the ascorbic acid concentration of urine can be made by using filter paper into which powdered indicator has been thoroughly rubbed. Strips of such paper dipped into urine samples give evidence of the presence or absence of appreciable amounts of ascorbic acid by their rate of decoloration.<sup>8</sup>

## ASCORBIC ACID CONTENT OF BLOOD

In estimating the vitamin C content of blood plasma 7 cc. of plasma are required. This is placed in a test tube and 3 cc. of 20 per cent trichloracetic acid is added. The tube is stoppered and well mixed, then centrifuged rapidly for ten

<sup>&</sup>lt;sup>8</sup> Scheer, K., Münch. med. Wchnschr., 85: 256, 1938.

minutes. The clear fluid is removed and the dye run in. A blank is run at the same time (diluted trichloracetic acid) and the amount of dye required to give the pink color is subtracted from the dye required by the blood sample. The vitamin C content is calculated directly, each cubic centimeter of blood filtrate representing 0.7 cc. of plasma.

Micro-Method of Farmer and Abt. We have also used this method with success. It is helpful when the blood samples are necessarily small. The instrument used in titrating is shown in Plate XLI. It is identical with that devised by Farmer and Abt with the exception of the screw controlled plunger which we have found more easily manipulated than the rubber sac used by Farmer and Abt. The technique is as follows:

Collect about 0.3 cc. blood from a lancet wound in a small phial containing potassium oxylate and stir. Centrifuge for a few minutes. Pipette 0.1 cc. plasma into a 15 cc. centrifuge tube and add 0.1 cc. distilled water, thereby rinsing the pipette. Add 0.2 cc. freshly prepared 5 per cent metaphosphoric acid solution, mix and then centrifuge.

The special pipette contains 0.1 cc., each scale division being 0.002 cc. The tip is bent down and ground to a point which is filmed with vaseline before using. The dye is dispensed from this pipette and the deproteinized plasma is placed in a depression on a white tile (0.2 cc. plasma). A blank is run with 0.1 cc. 5 per cent metaphosphotic acid solution plus 0.1 cc. distilled water. The end point for both is the first permanent pink. Calculations and preparation of the dye are similar to those used in the macro method.

# ASCORBIC ACID DETERMINATION BY METHOD OF EVELYN

2,6-dichlorophenolindophenol can be very successfully used in an electric photometer. The method of Evelyn is satis-

<sup>&</sup>lt;sup>9</sup> Farmer, C. J., and Abt., A. F., Proc. Soc. Exper. Biol. & Med., 34: 146, 1936.



PLATE XLI. A microburette suitable for estimating the vitamin C content of small amounts of blood. Excepting for the screw controlled plunger it is identical with the instrument designed by Farmer and Abt (see text).



factory. The dye is prepared so that 9 cc. plus 1 cc. of 5 per cent acetic acid reads approximately 30. Such solutions are filtered, adjusted to pH 7 with M 15 phosphate buffer and stored in brown bottles with glass stoppers. The instrument galvanometer is adjusted to 100 with a water blank, and a reading taken of 9 cc. plus 1 cc. 5 per cent acetic acid. This is L<sub>1</sub>. Adjust the galvanometer to 100 with blank containing 9 cc. water and 1 cc. urine and replace tube with one containing 1 cc. urine. Quickly run in 9 cc. of dye and read galvanometer at 5, 10 and 30 seconds. These values are plotted against time on graph paper and the curve extrapolated by free hand to intersect the axis of the ordinates. The calculated value is L<sub>2</sub>. The concentration of ascorbic acid = K (L<sub>1</sub> - L<sub>2</sub>)/A. The constant K must be determined for each photometer using pure ascorbic acid. "A" is the amount of urine used expressed in cubic centimeters.

Photometric determinations are extremely practical. In the determination of ascorbic acid in tissues and foods metaphosphoric acid should be used, largely because it inhibits copper-catalysis. The use of metaphosphoric acid in extracting the vitamin and an electric photometer to make feasible determinations within 5 to 10 seconds (which avoids interfering substances) constitutes a very practical test. As Bessey has pointed out determinations can be made even of colored solutions, such as beets. Furthermore it is possible to dispense with the uncertainty of determining the end point since the amount of decolorization of the dye is the criterion used.<sup>10</sup>

### APPLICATION TO SPECIAL PROBLEMS

The ascorbic acid concentration of animal tissues can best be determined if the tissues are ground in a mortar surrounded by dry ice (Muselin, Silverblatt, King and Woodward).

<sup>10</sup> Bessey, O. A., J. Biol. Chem., 126: 771, 1938. Evelyn, K. A., Malloy, H. T., and Rosen, C., J. Biol. Chem., 126: 645, 1938.

A special container surrounded by ice was used by Knight, Dutcher and Guerrant to collect milk for vitamin C determinations. The bottle was flushed with carbon dioxide and applied to the teat.

The fecal content of ascorbic acid may be determined if two determinations are made of a H<sub>2</sub>S free aqueous extract, one before and the other after oxidation of the ascorbic acid

by ascorbic acid oxidase (Chinn and Farmer).

Torrance devised a special technique for skin samples. The collagen was softened by heating with acetic and metaphosphoric acid in a sealed, evacuated tube. Thereafter the handling was as for blood plasma.<sup>11</sup>

# THE ESTIMATION OF CAPILLARY RESISTANCE

The resistance or fragility of the capillaries may be estimated by several methods.

Compression Test. The simplest procedure is to pinch a rectangular area of skin between the thumbs and forefingers of both hands and to note, after a period of one minute, whether petechiae are present and how numerous they are. This is a perfectly satisfactory method for establishing the presence of distinctly low capillary resistance.

Göthlin's Method. A popular procedure has been the Rumpel-Leede test in which the pressure within the capillaries is increased by constricting the arm veins. Göthlin modified the test and standardized the results. It requires a rubber arm band of the kind used with sphygmomanometers which is placed about the arm and inflated to a pressure less than the diastolic pressure of the pulse. Göthlin uses three pressures, 35, 50 and 60 mm. Hg to make the test more quantitative. The pressure is maintained for fifteen minutes.

<sup>Muselin, R. R., Silverblatt, E., King, C. G., and Woodward, G. E.,
Am. J. Cancer, 27: 707, 1936. Knight, C. A., Dutcher, R. A., and Guerrant,
N. B., Science, 89: 183, 1939. Chinn, H., and Farmer, C. J., Proc. Soc. Exper.
Biol. & Med., 41: 561, 1939. Torrance, C. C., Science, 87: 332, 1938.</sup> 

To interpret the test a circular area is imprinted on the skin over the antecubital fossa with a rubber stamp and the number of petechiae in this area are counted. Using a circle of 60 mm. diameter Göthlin found that healthy Scandinavians had fewer than five petechiae when a pressure of 50 mm. Hg was used. More than eight petechiae are considered subnormal.<sup>12</sup>

Suction Cup Method. Negative pressure outside the capillaries may be used instead of heightened pressure within. By this means the pressure range may be increased and more quantitative results are possible.

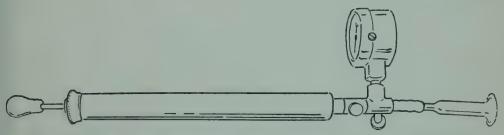


Fig. 28. A capillary resistometer using a spring manometer. The small screw projecting from below the dial is used to relieve the negative pressure at the end of the test period. (Drawing by permission of R. Blair.)

The test may be conducted in a number of ways. A satisfactory method is to use a small bicycle pump connected to a manometer and a small glass cup which is placed on the skin area to be tested and by means of which various negative pressures may be exerted on the capillaries. Since the capillary resistance varies in different regions of the body a small cup is preferable for a number of tests can be made in approximately the same region. We have used a cup with an inside diameter of 1 cm. and with a broad, flanged edge to prevent slipping of the skin.

The area of choice is the lateral aspect of the arm (not forearm) where the average resistance is approximately 30 cc.

<sup>12</sup> Göthlin, G. F., Skand. Arch. f. Physiol., 61: 225, 1931.

Hg, the center of the range of pressures possible with a hand pump. The cup is applied for one minute and the least negative pressure required to produce petechiae is determined

by testing at several levels.

A practical method of reading the results is to determine the pressure necessary to produce several macroscopic petechiae easily seen with the unaided eye by ordinary light. By using a hand glass and by blanching the skin with a glass slide hemorrhages may frequently be found at lesser pressures but they are often difficult to identify positively. The simpler method of reading the test has therefore seemed to us to be more definite. The results should be verified by testing at higher and lower pressures.<sup>13</sup>

#### DETERMINATION OF PROTHROMBIN

Quick's method seems best suited to most clinical problems. Four and one-half cubic centimeters venous blood is mixed with 0.5 cc. of sodium oxylate solution (1.34 gram anhydrous sodium oxylate per 100 cc. water) and centrifuged. Onetenth cubic centimeter of plasma so obtained is mixed with an equal amount of thromboplastin solution and finally with 0.1 cc. of calcium chloride solution (1.11 grams anhydrous calcium chloride per 100 cc. distilled water). The time required for the formation of a clot after the calcium chloride solution is added is measured with a stopwatch. Normal plasma clots in 12 to 13 seconds. Various workers have found normal individuals with much longer clotting time. Very extensive testing of nearly a hundred persons revealed 25 to 30 seconds represents the upper limits of normality (Mason). Hemorrhage does not occur when the clotting time is 20 seconds or less. Hemorrhage may occur if the clotting time is between 45 and 100 seconds (Snell, Butt and Osterberg). The explanation of this wide range of clotting time without a bleeding tendency is that the critical level of prothrombin concentra-

<sup>18</sup> Dalldorf, G., Am. J. Dis. Child., 46: 794, 1933.

tion is only 20 to 30 per cent of normal. As pointed out in Chapter XXII this represents a balance between rapid formation and destruction and is very unstable in patients with jaundice or hepatic diseases. Unfortunately the critical level cannot regularly be recognized by the clotting time test. Magath's suggestion of diluting the plasma with an equal amount of physiological saline and repeating tests in all cases in which the clotting time is 40 seconds or less is useful. If the clotting time of the diluted plasma is not greatly increased the prothrombin concentration is not seriously reduced. Where there is any doubt of the patients safety treatment should be relied on rather than the results of the determination of clotting time.

A reliable thromboplastin solution is necessary. Quick recommended macerating portions of a rabbits brain with acetone and repeating the process until a dry granular powder was obtained. This could be stored for a week in a refrigerator without loss of potency. The solution was prepared by dissolving 0.3 gram of the powdered brain tissue in 5 cc. physiologic saline, incubating for 10 minutes at 45°C. and centrifuging slowly for 3 minutes.

We have preserved thromboplastic preparations for several months by dessication in a lyophile apparatus. Such preparations will probably be marketed by biological supply houses and should greatly simplify the Quick test. The limitations of the test have been referred to in the text.

Kato and Poncher have used this method in determining the clotting rates of infant's blood removed by means of a puncture wound of the heel and using one-tenth as much plasma, thromboplastin and calcium chloride solutions.<sup>14</sup>

A simple determination can be made using whole blood.

<sup>14</sup> Quick, A. J., J. A. M. A., 110: 1658, 1938. Smith, H. P., Ziffren, S. E., Owen, B. A. and Hoffman, G. R., J. A. M. A., 113: 380, 1939. Kato, K., and Poncher, H. G., J. A. M. A., 114: 749, 1940. Warner, E. D., Brinkhous, K. M., and Smith, H. P., Am. J. Physiol., 114: 667, 1936. Magath, T. B., Proc. Staff Meet. Mayo Clinic, 13: 67, 1938.

The sample (0.7 cc.) is discharged directly into a test tube containing 0.1 cc. of thromboplastin solution, the tube inverted once and tilted thereafter to reveal the time required for the formation of a clot. A normal blood should be timed as a control. The results are expressed as "clotting activity" which is expressed in per cent of normal. Values below 100 per cent indicate a bleeding tendency, hemorrhage will occur at values between 30 to 50 per cent (Smith, Ziffren, Owen and Hoffman).

The translation of clotting time into prothrombin values is facilitated by Quick's chart which is reproduced.

The two-stage method of Warner, Brinkhouse and Smith is not adapted to clinical use. The authors' report should be consulted.

# Urinary Excretion of Vitamin B<sub>6</sub><sup>15</sup>

The urine is made strongly alkaline (above pH 9.8 using thymol blue as indicator) with 30 per cent sodium hydroxide and allowed to stand overnight to destroy interfering reducing substances. The following morning 2 cc. samples are removed, about 45 cc. of water and 2 drops of half normal acetic acid are added. Adjust the pH to between 7.0 and 7.6 using phenol red on a spot plate. The volume is then brought to 50 cc.

To 5 cc. of the adjusted and diluted urine add 5 cc. of veronal buffer and 20 cc. of the chloramide in butanol solution. Stopper and shake vigorously. Allow to stand for 5 minutes and shake again. Allow to stand for 10 minutes. Centrifuge (three minutes will separate the layers). Wash the butanol layer twice with 10 cc. portions of the veronal buffer separating the layers by centrifugalization as above.

To 15 cc. of the washed butanol layer add 5 cc. of absolute alcohol, mix and read in the colorimeter. Our determinations have been made in a Cenco photometer using \*4 filter

<sup>&</sup>lt;sup>15</sup> Scudi, J. V., Unna, K., and Antopol, W., J. Biol. Chem., 135: 371, 1940.

and adjusting the instrument against a blank prepared from the patient's urine collected before administering vitamin B<sub>6</sub>. This requires that a comparable urine sample be collected before administering the vitamin. Hourly specimens are therefore collected, the first one hour after the fasting patient has taken 250 cc. of water. This is discarded and a second sample collected one hour later during which time the patient drinks another 250 cc. of water. Three hourly samples are collected after administering the vitamin. The excretion peak is reached within the first hour and falls rapidly after the second hour. If large amounts of vitamin be administered (5 mgm. per kgm.) the excretion within 3 hours may exceed 25 per cent of the intake.

# APPENDIX B THE VITAMIN CONTENT OF FOODS

Vitamin A Content of Foods

| FOOD                    | VITAMIN A    | REFERENCE |
|-------------------------|--------------|-----------|
| Meat:                   |              |           |
| Bacon                   | 25           | (1)       |
| Beef, average lean, raw | 56           | (2)       |
| Kidney, veal            | 1,000        | (1)       |
| Liver, beef             | 9,000        | (1)       |
| Liver, calf             | 5,475        | (2)       |
| Liver, lamb             | 5,475        | (2)       |
| Liver, pork             | 6,000        | (2)       |
| Fish and shellfish:     |              |           |
| Cod                     | 5            | (1)       |
| Haddock                 | 5            | (1)       |
| Salmon                  | 30-750       | (1)       |
| Clams                   | 14           | (1)       |
| Oysters                 | 140          | (1)       |
| Liver oils:             |              |           |
| Bluefin tuna            | 78,000       | (6)       |
| Striped tuna            | 33,000       | (6)       |
| Shark                   | 5,500-12,000 | (6)       |
| Totoaba                 | 45-80,000    | (6)       |
| Mackerel                | 30-200,000   | (6)       |
| Mackerel body           | 700          | (6)       |
| Dairy products:         |              |           |
| Butter, fresh           | 2,400        | (1)       |
| Cheddar                 | 2,000        | (1)       |
| Cottage                 | 500          | (1)       |
| Cream                   | 2,100        | (1)       |
| Cream, 20 per cent      | 680          | (1)       |
| Cream, 40 per cent      | 2,160        | (7)       |
| Egg, whole, hen         | 1,000        | (1)       |
| Egg, white of, raw      | 0            | (1)       |

<sup>\*</sup> Respectively.

| FOOD                           | VITAMIN A      | REFERENCE |
|--------------------------------|----------------|-----------|
| Dairy products—Continued       |                |           |
| Egg, yolk of, raw              | 9 900          | (4)       |
| Milk, whole, cow               | 2,800          | (1)       |
| Milk, condensed and evaporated | 110<br>670     | (1)       |
| Milk, whole, dried             | 875            | (7)       |
| Milk, skim                     |                | (1)       |
| Milk, malted                   | 2              | (1)       |
| Milk, human                    | $4,500 \\ 110$ | (8)       |
| Fruit:                         |                |           |
| Apples, average                | 75             | (1)       |
| Apricots, fresh                | 4,000          | (1)       |
| Apricots, dried                | 5,000          | (1)       |
| Avocados, average              | 100            | (1)       |
| Bananas, Gros Michel           | 300            | (1)       |
| Blackberries, average          | 150            | (1)       |
| Blueberries, average           | 100            | (1)       |
| Cantaloupe, average            | 300            | (1)       |
| Cherries, Royal Anne.          | 200            | (1)       |
| Cranberries, average           | 200            | (1)       |
| Dates, dried                   | 150            | (1)       |
| Figs, fresh                    | 50             |           |
| Figs, dried                    | 60             | (1)       |
| Grapes, average                | trace          | (1)       |
| Grapefruit, Florida            | 0              | (9)       |
| Lemon                          | 0              | (1)       |
| Lemon juice                    | 0              | (1)       |
| Mango                          | 1,500          | (1)       |
| Nectarine                      | 2,900          | (10)      |
| Olives, green                  | 190            | (1)       |
| Olives, ripe                   | 125            | (1)       |
| Orange, California             | C E            | (9)       |
| Orange juice                   | 45-350         | (1)       |
| Peaches, yellow                | 1,000          | (1)       |
| Peaches, dried, yellow         | 3,000          | (1)       |
| Pears, average                 | 10             | (1)       |
| Pineapple                      | 90             | (1)       |
| Pineapple juice                | 147            | (11)      |
| Prunes, dried                  | 2,500          | (1)       |
| Raspberries.                   | 520            | (9)       |
| Strawberry                     | 740            | (9)       |
| Watermelon                     | 125            | (10)      |
| Youngberry                     | 460            | (9)       |
| 20003                          | 100            | (0)       |

| FOOD                       | VITAMIN A | REFERENCE |
|----------------------------|-----------|-----------|
| Nuts:                      |           |           |
| Pecan                      | 400       | (1)       |
| Vegetables:                |           |           |
| Artichokes                 | 200       | (1)       |
| Asparagus, green           | 700       | (1)       |
| Asparagus, white           | 0-50      | (1)       |
| Beans:                     |           |           |
| String                     | 1,000     | (1)       |
| Navy, dry                  | 74        | (10)      |
| Lima, green                | 500       | (1)       |
| Soy, fresh, green          | 200       | (1)       |
| Green, snap                | 1,000     | (1)       |
| Soy, dried                 | 100       | (1)       |
| Beets, root                | trace     | (1)       |
| Broccoli, green            | 9,000     | (1)       |
| Brussel sprouts, fresh     | 200       | (1)       |
| Cabbage, head              | 0-100     | (1)       |
| Carrot, fresh              | 2,100     | (1)       |
| Cauliflower, fresh         | 30        | (1)       |
| Celery, bleached           | 10        | (1)       |
| Chard, fresh               | 9,000     | (1)       |
| Collards, fresh            | 7,000     | (1)       |
| Corn, yellow, sweet, fresh | 500       | (1)       |
| Corn, Golden Bantam        | 10-20     | (12)      |
| Cucumber, green            | 20        | (1)       |
| Egg plant, fresh           | 35        | (1)       |
| Endive, fresh              | 15,000    | (1)       |
| Escarole, fresh            | 15,000    | (1)       |
| Greens:                    | 10,000    | (1)       |
| Dandelion                  | 12,000    | (1)       |
| Turnip                     | 10,000    | (1)       |
| Kale, fresh                | 20,000    |           |
| Lettuce, green             | 4,000     | (1)       |
| Lettuce, head              | 100       | (1)       |
| Mushrooms, fresh           | 0         | . ,       |
| Okra, fresh                | 400       | (1)       |
| Onions, raw                | 0         | (1)       |
| Parsley, fresh             | 30,000    | (1)       |
| Parsnips, roots            | trace     | (1)       |
| Peas, green, raw.          |           | (1)       |
| Peas, cowpeas, dried.      | 1,000     | (1)       |
| F-001 database             | 50        | (1)       |

| FOOD                              | VITAMIN A | REFERENCE |
|-----------------------------------|-----------|-----------|
| Vegetables—Continued              |           |           |
| Peas, Thomas Laxton               | 610-620   | (13)      |
| Peppers, red                      | 5,000     | (1)       |
| Peppers, green                    | 5,000     | (1)       |
| Potatoes, sweet, raw              | 3,500     | (1)       |
| Potatoes, white, raw, new Cobbler | 30        | (1)       |
| Pumpkin, raw                      | 2,500     | (1)       |
| Rhubarb, raw                      | trace     | (1)       |
| Romaine, raw                      | 800       | (1)       |
| Rutabaga, white, raw              | 0         | (1)       |
| Rutabaga, yellow, raw             | 25        | (1)       |
| Sauerkraut, fresh                 | 25        | (1)       |
| Spinach, raw                      | 25,000    | (1)       |
| Squash, winter, raw, Hubbard      | 4,000     | (1)       |
| Tomato, raw                       | 1,000     | (1)       |
| Tomato juice                      | 1,000     | (1)       |
| Turnip, white, raw                | 0         | (1)       |
| Miscellaneous:                    |           |           |
| Breads:                           |           |           |
| Whole wheat                       | trace     | (1)       |
| White wheat                       | trace     | (1)       |
| Whole grains:                     |           |           |
| Barley                            | 0         | (1)       |
| Wheat                             | trace     | (1)       |
| Rice, brown                       | trace     | (1)       |
| Oats                              | trace     | (1)       |
| Rye                               | 0         | (1)       |
| Rice, white                       | 0         | (1)       |
| Flours:                           |           | (4)       |
| Patent white wheat                | 0         | (1)       |
| Rye                               | 0         | (1)       |
| Meals:                            |           | (4)       |
| White corn                        | 0         | (1)       |
| Yellow corn                       | 500       | (1)       |
| Farina                            | 0         | (1)       |
| Hominy, yellow                    | 600       | (14)      |
| Hominy, white                     | . 0       | (14)      |
| Pastes:                           |           | (1)       |
| Macaroni                          | 0         | (1)       |
| Brans and cereal coats:           |           | (4)       |
| Wheat bran                        | 140       | (1)       |
| Rice polish                       | 0         | (14)      |

| FOOD                    | VITAMIN A      | REFERENCE |
|-------------------------|----------------|-----------|
| Miscellaneous—Continued |                |           |
| Lard, hog               | 7              | (15)      |
| Oils:                   |                |           |
| Corn                    | 0              | (1)       |
| Cottonseed              | 0              | (1)       |
| Coconut                 | 0              | (1)       |
| Olive                   | . 0            | (1)       |
| Sardine body            | 300            | (6)       |
| Salmon liver            | 5,800-57,000   | (6)       |
| Salmon body             | 525            | (6)       |
| White sea bass liver    | 45,000-180,000 | (6)       |
| Bluefin tuna liver      | 78,000         | (6)       |
| Bluefin tuna offal oil  | 203-720        | (6)       |
| Mackeral liver          | 16,000-211,000 | (6)       |
| Shark liver             | 5,500-120,000  | (6)       |
| Yeast:                  |                |           |

# Vitamin B<sub>1</sub> Content of Foods

| FOOD                | VITAMIN B1            | REFERENCE |
|---------------------|-----------------------|-----------|
| Meat:               |                       |           |
| Bacon, average      | 33                    | (18)      |
| Beef, lean, muscle  | 25-38                 | (2)       |
| Brain, beef         | 56                    | (2)       |
| Chicken, average    | 43                    | (2)       |
| Chicken, white meat | 25-48                 | (2)       |
| Chicken, dark meat  | <b>5</b> 9- <b>77</b> | (2)       |
| Ham, fresh          | 303-510               | (2)       |
| Ham, smoked         | 358-476               | (2)       |
| Ham, canned         | 295                   | (17)      |
| Heart, beef         | 225                   | (2)       |
| Heart, mutton       | 150                   | (2)       |
| Heart, pork         | 174                   | (2)       |
| Kidney, beef        | 105                   | (2)       |
| Kidney, veal        | 60                    | (2)       |
| Lamb, average lean  | 60-111                | (2)       |
| Liver, beef         | 89-129                | (2)       |
| Liver, calf         | 45                    | (2)       |
| Liver, lamb         | 1 00-138              | (2)       |
| Liver, pork         | 156                   | (2)       |
| Lung, beef          | 70                    | (16)      |
| Pork, average lean  | 303-510               | (2)       |

| FOOD                           | VITAMIN B1 | REFERENCE |
|--------------------------------|------------|-----------|
| Meat—Continued                 |            |           |
| Pork, chop                     | 455        | (4)       |
| Sausage, pork                  | 115        | (2)       |
| Sausage, bologna               | 175        | (2)       |
| Spleen, beef                   | 66         | (16)      |
| Tongue, beef                   | 100        | (17)      |
| Veal, average lean, raw        | 40-112     | (17)      |
| Fish and shellfish:            |            |           |
| Average, lean                  | 13         | (18)      |
| Cod                            | 30         | (1)       |
| Haddock                        | 5          | (1)       |
| Herring                        | 35         | (18)      |
| Salmon                         | trace      | (1)       |
| Salmon, red, canned            | trace      | (4)       |
| Halibut                        | 28         | (4)       |
| Trout                          | 29         | (4)       |
| Clams, average                 | 7          | (1)       |
| Oysters, average               | 75         | (14)      |
| Dairy products:                |            |           |
| Butter, fresh                  | 35         | (7)       |
| Cheese, Cheddar                | 14         | (4)       |
| Cream, 20 per cent             | 10         | (7)       |
| Egg, whole, hen                | 50         | (18)      |
| Egg, white of, raw             | trace      | (4)       |
| Egg, yolk of, raw              | 118        | (4)       |
| Milk, whole, cow               | 16         | (4)       |
| Milk, condensed and evaporated | 24         | (4)       |
| Milk, whole, dried             | 105        | (4)       |
| Milk, skim                     | 14         | (4)       |
| Milk, buttermilk               | 2          | (7)       |
| Milk, malted                   | 100-120    | (8)       |
| Milk, human                    | 10-12      | (5)       |
| Milk, evaporated               | 17         | (4)       |
| Milk, skim, dried              | 125        | (4)       |
| Fruits:                        |            |           |
| Apples, average                | 15         | (1)       |
| Apples, Stayman Winesap        | 8          | (4)       |
| Apricots, fresh                | 10         | (1)       |

| FOOD                            | VITAMIN B1 | REFERENCE |
|---------------------------------|------------|-----------|
| Fruits—Continued                |            |           |
| Apricots, dried                 | 30         | (1)       |
| Avocados, average               | 34         | (4)       |
| Bananas, Gros Michel            | 14-18      | (19)      |
| Bananas, yellow peel, green tip | 18         | (4)       |
| Blackberries, average           | 8          | (4)       |
| Blueberries, average            | 15         | (4)       |
| Cantaloupe, average             | 20         | (1)       |
| Cherries, Bing                  | 17         | (4)       |
| Coconut, average                | 20         | (1)       |
| Dates, dried                    | 25         | (1)       |
| Dates, Hayany                   | 24         | (4)       |
| Figs, fresh                     | 25         | (1)       |
| Figs, dried                     | 22         | (1)       |
| Grapes, average                 | 15         | (1)       |
| Grapefruit, Florida             | 23         | (1)       |
| Grapefruit, fresh               | 24         | (4)       |
| Lemon                           | 10         | (1)       |
| Lemon juice                     | 10         | (1)       |
| Muskmelon, average              | 19         | (4)       |
| Olives, ripe                    | 2          |           |
| Orange                          | 26         | (1)       |
| Orange juice                    | 30         | (4)       |
| Peaches, yellow                 | 10         | (1)       |
| Peaches, dried, yellow          | 17         | (1)       |
| Peaches, white, fresh           | 8          | (1)       |
| Pears, average                  | 8          | (4)       |
| Pineapple                       | 30         | (4)       |
| Pineapple juice                 | 25         | (4)       |
| Plum, Texas                     | 25<br>16   | (11)      |
| Prunes, dried                   | 60         | (4)       |
| Raspberry, black                |            | (4)       |
| Raspberry, red                  | 8          | (4)       |
| Strawberry                      | 8          | (4)       |
| Watermelon                      | 8          | (4)       |
|                                 | 20         | (1)       |
| Nuts:                           |            |           |
| Almonds                         | 75         | (1)       |
| Chestnuts                       | 57         | (1)       |
| Filberts                        | 206        | (18)      |

| FOOD                       | VITAMIN B1 | REFERENCE |
|----------------------------|------------|-----------|
| Vuts—Continued             |            |           |
| Hazel                      | 220        | (1)       |
| Peanuts                    | 250-350    | (4)       |
| Pecan                      | 350        | (1)       |
| Walnut                     | 114        | (4)       |
| Peanut skins               | 2,632      | (4)       |
| Peanut germ                | 294        | (4)       |
| Peanuts, wasted            | <b>7</b> 8 | (4)       |
| Vegetables:                |            |           |
| Artichokes, average        | 60         | (1)       |
| Asparagus, green           | 70         | (1)       |
| Asparagus, white           | 50         | (1)       |
| Asparagus                  | <b>5</b> 9 | (4)       |
| Beans:                     |            |           |
| String                     | 25         | (1)       |
| Navy, dry                  | 128        | (4)       |
| Kidney, dry                | 150        | (1)       |
| Lima, green                | 114        | (4)       |
| Lima, dried                | 170        | (4)       |
| Green snap                 | 24         | (4)       |
| Yellow wax                 | 29         | (4)       |
| Soy, fresh, green          | 175        | (1)       |
| Soy, dried                 | 485        | (4)       |
| Beet root                  | 17         | (4)       |
| Broccoli, green            | 33         | (4)       |
| Brussel Sprouts, fresh     | 57         | (4)       |
|                            | 27         | (4)       |
| Cabbage, head              | 24         | (4)       |
| Carrot, fresh              | 56         | (4)       |
| Cauliflower                | 12         | (4)       |
| Celery, bleached           | 67         | (4)       |
| Collards, fresh            | 45         | (1)       |
| Corn, yellow, sweet, fresh | 40         | (4)       |
| Corn, Country Gentlemen    | 50         | (4)       |
| Corn, Golden Bantam        | 15         | (1)       |
| Cucumber, green            | 15         | (1)       |
| Egg plant, fresh           | 33         | (4)       |
| Endive, fresh              | 00         |           |
| Greens:                    | 46         | (4)       |
| Mustard                    | 46         | (4)       |
| Turnip                     | 40         | (1)       |

| FOOD                              | VITAMIN B1 | REFERENCE |
|-----------------------------------|------------|-----------|
| Vegetables—Continued              |            |           |
| Kale, fresh                       | 63         | (4)       |
| Kohlrabi, fresh                   | 20         | (1)       |
| Leek                              | 50         | (1)       |
| Lentil                            | 170        | (1)       |
| Lettuce, green                    | 25         | (1)       |
| Lettuce, head                     | 29         | (4)       |
| Mushrooms, fresh                  | 30         | (1)       |
| Okra, fresh                       | 42         | (4)       |
| Onions, raw                       | 10         | (4)       |
| Parsnips, roots                   | 40         | (1)       |
| Peas, green, raw                  | 140        | (1)       |
| Peas, cowpeas, dried              | 312        | (4)       |
| Peppers, red                      | 10         | (1)       |
| Peppers, green                    | 10         | (1)       |
| Potatoes, sweet, raw              | 31         | (4)       |
| Potatoes, white, raw, new Cobbler | 62         | (4)       |
| Potatoes, white, raw, old Cobbler | 49         | (4)       |
| Pumpkin, raw                      | 15         | (1)       |
| Radish, raw                       | 20         | (1)       |
| Rhubarb, raw                      | 8          | (4)       |
| Rutabaga, white, raw              | 15         | (1)       |
| Rutabaga, yellow, raw             | 25         | (4)       |
| Sauerkraut, fresh                 | 10         | (4)       |
| Spinach, raw                      | 35         | (4)       |
| Squash, winter, raw, Hubbard      | 16         | (4)       |
| Summer squash, raw                | 14         | (4)       |
| Tomato, raw                       | 20-26      | (4)       |
| Tomato juice                      | 20-26      | (1)       |
| Tomato, red                       | 26         | (4)       |
| Tomato, white                     | 20         | (4)       |
| Turnip, white, raw                | 20         | (4)       |
| Watercress                        | 40         | (1)       |
| Miscellaneous:                    |            |           |
| Bread:                            |            |           |
| Wholewheat                        | 133        | (18)      |
| White wheat                       | 15         | (18)      |
|                                   | 10         | (10)      |

| FOOD                    | VITAMIN B1 | REFERENCE |
|-------------------------|------------|-----------|
| Miscellaneous-Continued |            |           |
| Whole grains:           |            |           |
| Barley                  | 120        | (1)       |
| Corn, whole kernel      | 120        | (1)       |
| Wheat                   | 118-175    | (20)      |
| Rice, brown             | 159-175    | (20)      |
| Rice, white             | 100 110    | (18)      |
| Oats                    | 210-228    | (20)      |
| Rye                     | 131-156    | (4)       |
| Buckwheat               | 66         | 1 ' '     |
| Shredded wheat          | 73         | (18)      |
| Flours:                 | 10         | (4)       |
| Patent white wheat      | 17         | (4)       |
| Whole wheat             | 160–190    | (20)      |
| Graham                  | 110-150    | (10)      |
| Rye                     | 56         | (18)      |
| Straight milled wheat   | 29         |           |
| White, "plus germ"      | 43         | (4)       |
| Meals:                  | 40         | (4)       |
| White corn              | 101        | (4)       |
| Yellow corn             | <b>7</b> 8 | (4)       |
| Farina                  | 24-43      | (20)      |
| Hominy, yellow          | 66         | (18)      |
| Hominy, white           | 66         | (18)      |
| Oatmeal                 | 190        | (20)      |
| Oatmeal, Quick.         | 270        | (4)       |
| Rolled                  | 242        | (4)       |
| Corn Flakes             | trace      | (4)       |
| Pastes:                 | Grace      | (4)       |
| Macaroni                | 16         | (18)      |
| Sago                    | 3          | (18)      |
| Tapioca                 | 3          | (18)      |
| Cereal embryo:          | 0          | (10)      |
| Wheat                   | 600-1,333  | (18)      |
| Oat                     | 000 1,000  | (10)      |
| Brans and cereal coats: |            |           |
| Rice polish             | 666-1,250  | (18)      |
| Wheat bran              | 100-360    | (10)      |
| Lard, hog               | 33         | (18)      |
| Molasses, sugar cane    | trace      | (4)       |
| Mustard, ordinary       | 3          | (18)      |
| Musualu, orumary        | U          | (10)      |

| FOOD                    | VITAMIN B1 | REFERENCE |
|-------------------------|------------|-----------|
| Miscellaneous—Continued |            |           |
| Oils:                   |            |           |
| Corn                    | 0          | (1)       |
| Cottonseed              | 0          | (1)       |
| Coconut                 | 0          | (1)       |
| Olive                   | 0          | (1)       |
| Yeast:                  |            |           |
| Brewers'                | 666        | (18)      |
| Bakers'                 | 233        | (18)      |

# Vitamin G Content of Foods

| FOOD                    | VITAMIN G             | REFERENCE |
|-------------------------|-----------------------|-----------|
| Meat:                   | micrograms riboflavin |           |
| Bacon                   | 30                    | (21)      |
| Beef, average lean, raw | 375                   | (2)       |
| Ham, fresh              | 195-300               | (2)       |
| Ham, smoked             | 156-300               | (2)       |
| Heart, beef             | 767-900               | (2)       |
| Kidney, beef            | 1,872-2,400           | (2)       |
| Kidney, veal            | 2,400-2,700           | (2)       |
| Liver, beef             | 3,000-3,700           | (2)       |
| Liver, calf             | 2,700-3,900           | (2)       |
| Liver, lamb             | 2,610-2,990           | (2)       |
| Liver, pork             | 2,625-2,900           | (2)       |
| Pork, average lean      | 287                   | (2)       |
| Veal, average lean, raw | 345                   | (2)       |
| Fish:                   |                       |           |
| Salmon                  | 275                   | (1)       |
| Dairy products:         |                       |           |
| Butter, fresh           | 0                     | (1)       |
| Cheese:                 |                       | (-)       |
| Cheddar                 | 750                   | (10)      |
| Cream                   | 138                   | (22)      |
| Swiss                   | 200                   | (22)      |
| Egg, hen, whole         | 330                   | (1)       |
| Egg, white of, raw      | 300                   | (1)       |
| Egg, yolk of, raw       | 345                   | (1)       |
| Milk, whole, cow        | 225                   | (7)       |

| FOOD                                   | VITAMIN G             | REFERENCE |
|--|-----------------------|-----------|
| Dairy products—Continued               | micrograms riboflavin |           |
| Milk, whole, condensed and evaporated. | 303                   | (7)       |
| Milk, whole, dried                     |                       | (1)       |
| Milk, skim                             |                       | (1)       |
| Milk, buttermilk                       |                       | (17)      |
| Milk, malted                           |                       | (10)      |
| Fruit:                                 |                       |           |
| Apples, average                        | 73                    | (3)       |
| Apricots, fresh                        | 51                    | (1)       |
| Apricots, dried                        | 105                   | (1)       |
| Avocados                               | 90                    | (1)       |
| Bananas, Gros Michel                   |                       | (3)       |
| Blueberries                            | _                     |           |
| Cantaloupe                             | <b>—</b>              | (3)       |
| Cherries, Royal Anne                   |                       | (1)       |
| Figs, fresh                            |                       | (1)       |
| Figs, dried                            |                       | (1)       |
| Grapes, average                        |                       | (3)       |
| Grapefruit, Florida                    |                       | (1)       |
| Mango                                  |                       | (1)       |
| Olives, ripe                           |                       | (1)       |
| Orange                                 |                       | (1)       |
| Orange juice                           | 4 44                  | (1)       |
| Pears, average                         | <b>—</b>              | (3)       |
| Pineapple                              | 0.0                   | (1)       |
| Pineapple juice                        | 01                    | (1)       |
| Prunes, dried                          | 1 1 1 1               | (1)       |
| Tangerine                              |                       | (1)       |
| Watermelon                             | 00                    | (1)       |
| Young berry                            |                       | (1)       |
| Nuts:                                  |                       |           |
| Peanut butter                          |                       | (10)      |
| Pecan                                  | 300                   | (1)       |
| Vegetables:                            | 100                   | (2)       |
| Asparagus, green                       |                       | (3)       |
| Beans, string                          | 93                    | (3)       |
| Beans, navy, dry                       | 1,200                 | (1)       |
| Beans, lima, green                     | 900                   | (1)       |
| Beans, soy, fresh green                | . 300                 | (1)       |

| FOOD                              | VITAMIN G             | REFERENCE |
|-----------------------------------|-----------------------|-----------|
| Vegetables—Continued              | micrograms riboflavin |           |
| Beets, root                       | 23                    | (3)       |
| Broccoli, green                   | 210                   | (3)       |
| Cabbage, head                     | 44                    | (3)       |
| Carrot, fresh                     | 66                    | (3)       |
| Cauliflower, fresh                | 127                   | (3)       |
| Collards, fresh                   | 300                   | (23)      |
| Escarole                          | 120                   | (1)       |
| Greens:                           |                       | , ,       |
| Beet                              | 82                    | (3)       |
| Mustard                           | 450                   | (10)      |
| Dandelion                         | 225                   | (10)      |
| Turnip                            | 360                   | (1)       |
| Kale, fresh                       | 600                   | (1)       |
| Kohlrabi, fresh                   | 75                    | (14)      |
| Lettuce, green                    | 71                    | (3)       |
| Lettuce, head                     | 48                    | (3)       |
| Mushrooms                         | 0                     | (1)       |
| Onions, raw                       | 123                   | (3)       |
| Peas, green, raw                  | 131-154               | (3)       |
| Peppers, red                      | 138                   | (3)       |
| Peppers, green                    | 138                   | (3)       |
| Potatoes, sweet, raw              | 68-70                 | (3)       |
| Potatoes, white, raw, new Cobbler | 44-55                 | (3)       |
| Spinach                           | 160                   | (3)       |
| Tomato, raw                       | 52                    | (3)       |
| Tomato juice                      | 36–174                | (1)       |
| Turnip, white, raw                | 42                    | (3)       |
| Watercress, raw                   | 270                   | (1)       |
| Bread:                            |                       |           |
| Whole wheat                       | 120                   | (10)      |
| Whole grains:                     |                       |           |
| Barley                            | 0                     | (1)       |
| Wheat                             | 105                   | (1)       |
| Rye                               | 105                   | (1)       |
| Flours:                           |                       |           |
| Patent white wheat                | 0                     | (1)       |
| Meals:                            |                       |           |
| Yellow corn meal                  | 99                    | (10)      |

| FOOD              | VITAMIN G             | REFERENCE |
|-------------------|-----------------------|-----------|
| Cereal embryo:    | micrograms riboflavin |           |
| Wheat             | 450-1,212             | (10)      |
| Oils:             |                       |           |
| Corn              | 0                     | (1)       |
| Cottonseed        | 0                     | (1)       |
| Coconut           | 0                     | (1)       |
| Olive             | 0                     | (1)       |
| Yeast:            |                       |           |
| Brewers', Bakers' | 750-7,500             | (10)      |
| Wines:            |                       |           |
| White             | 100-150               | (24)      |
| Red               | 27-90                 | (24)      |

# Nicotinic Acid Content of Food

| FOOD SOURCE       | MILLIGRAMS OF NICOTINIC ACID PER 100 GRAMS OF FOOD |       |
|-------------------|--|-------|
|                   | Dry  | Fresh |
| Meat:             |  |       |
| Pork liver        | 90.0   | 26.4  |
| Pork liver        | 110.0  | 27.5  |
| Beef liver        | 110.0  | 27.5  |
| Beef liver        | 85.0   | 25.0  |
| Beef liver, fried | 87.0   | 29.4  |
| Veal liver        | 72.0   | 22.5  |
| Lamb liver        | 131.0  | 46.0  |
| Lamb liver        | 135.0  | 39.2  |
| Pork kidney       | 72.0   | 15.5  |
| Beef kidney       | 81.0   | 16.9  |
| Beef kidney       | 89.0   | 17.8  |
| Pork heart        | 32.0   | 8.0   |
| Beef heart        | 23.0   | 4.9   |
| Beef spleen       | 28.0   | 7.0   |
| Beef spleen       | 33.0   | 8.3   |
| Beef spleen       | 52.0   | 12.3  |
| Pork ham          | 40.0   | 9.7   |

Reference: The Journal of Nutrition, Vol. 19, No. 5, May 10, 1940, p. 487.

| MILLIGRAMS OF NICE 100 GRAMS   |                 |                |
|--|-----------------|----------------|
| -  | Dry             | Fresh          |
| Meat—Continued   |                 |                |
| Pork ham   | 38.0            | 10.4           |
| Pork ham   | 37.0            | 10.0           |
| Pork ham   | 32.0            | 8.8            |
| Boiled ham   | 15.0            | 5.2            |
| Smoked ham   | 28.0            | 8.2            |
| Tenderized ham   | 25.0            | 8.3            |
| Pork loin.   | 46.0            | 13.0           |
| Pork loin.   | 25.0            | 7.5            |
| Pork loin.   | 19.0            | 5.3            |
| Beef tongue  | 46.0            | 12.8           |
| Veal hindquarter   | 70.0            | 16.1           |
| Veal hindquarter   | 72.0            | 18.0           |
| Veal hindquarter   | 24.0            | 6.5            |
| Beef brain   | 30.0            | 7.5            |
| Beef muscle.   | 15.0            | 3.8            |
| Beef muscle.   | 37.0            | 10.2           |
| Roast beef   | 37.0            | 10.2           |
| Beef pancreas  | 11.0            | 2.7            |
| Beef pancreas  | 16.0            | 3.5            |
| Beef lung  | 33.0            | 8.3            |
| FOODSTUFF  |                 | NICOTINIC ACID |
|  |                 | mgm, per cent  |
| Miscellaneous: Skim milk powder  |                 | 4.3-6.2        |
| Bakers yeast   | T T             | 50             |
| Brewers yeast, Sample A  |                 | 93             |
|  |                 | 90             |
|  |                 | 35             |
|  |                 | 34             |
|  |                 | 39             |
| $\mathbf{F}_{\cdots}$  | 56              |                |
| Liver extract, alcohol soluble (Wilson L                                       | aboratories no. |                |
| 36997)   | 00010           | 450            |
| Liver fraction "B" (Wilson Laboratories no. 38818)                             |                 | 210            |
| Liver extract powder (Wilson Laborato<br>Alcohol-ether precipitate fraction of | liver extract   | 270            |
| powder   |                 | Less than 35   |

Reference: The Journal of Nutrition, Vol. 19, No. 5, May 10, 1940, p. 489.

| FOODSTUFF                          | NICOTINIC ACID |
|------------------------------------|----------------|
|                                    | mgm. per cent  |
| Miscellaneous—Continued            |                |
| Rice bran extract, a commercial    | 165            |
| Whey concentrate, a commercial     | Less than 15   |
| Peanut meal (raw)                  | 13             |
| Grass, a commercial preparation    | 7.8            |
| Egg yolk (hard boiled)             | Less than 4    |
| Egg white (hard boiled)            | Less than 2.5  |
| Wheat germ                         | Less than 4    |
| Extracted wheat germ, a commercial | Less than 6    |

# Vitamin B<sub>6</sub> Content of Foods

| FOOD                    | VITAMIN B6 |  |
|-------------------------|------------|--|
| Meat:                   |            |  |
| Beef, average lean, raw | 25         |  |
| Liver, pork             | 100        |  |
| Fish:                   |            |  |
| Haddock                 | 40         |  |
| Dairy products:         |            |  |
| Butter, fresh           | 200        |  |
| Cheese, Cheddar         | 250        |  |
| Egg, yolk of, raw       | 2,500      |  |
| Milk, whole, cow        | 90         |  |
| Milk, skim              | 14         |  |
| Milk, skim, dried       | 56         |  |
| Fruit:                  |            |  |
| Apples, average         | 25         |  |
| Bananas, Gros Michel    | 66         |  |
| Orange, California      | 16         |  |
| Nuts:                   |            |  |
| Peanuts                 | 1,600      |  |
| Vegetables:             |            |  |
| Beans, navy, dry        | 400        |  |
| Beet, root              | 13         |  |
| Lettuce, green          | 25         |  |

| FOOD                              | VITAMIN B6 |
|-----------------------------------|------------|
| Vegetables—Continued              |            |
| Potatoes, white, raw, new Cobbler | 40         |
| Spinach                           | 66         |
| Tomato, raw                       | 25         |
| Miscellaneous:                    |            |
| Bread, whole wheat                | 400        |
| Cornmeal, yellow                  | 400        |
| Oatmeal                           | 330        |
| Cereal embryo:                    |            |
| Wheat                             | 1,250      |
| Brans and cereal coats:           |            |
| Rice polish                       | 500        |
| Lard:                             |            |
| Hog                               | 2,500      |
| Oils:                             |            |
| Corn                              | 2,000      |
| Peanut                            | 5,000      |
| Soy Bean                          | 1,250      |

Reference: Schneider, H. A., Ascham, J. K., Platz, B. B., and Steenbock, H. J. Nutrition 18: 99, 1939.

#### Vitamin C Content of Foods

| FOOD               | VITAMIN C | REFERENCE |
|--------------------|-----------|-----------|
| Meat:              |           |           |
| Bacon, average     | . 0       | (1)       |
| Beef, lean, raw    | 0         | (1)       |
| Liver, calf        | 200       | (2)       |
| Dairy products:    |           |           |
| Butter             | 0         | (25)      |
| Egg, whole, hen    | 0         | (25)      |
| Egg, white of, raw | 0         | (25)      |
| Egg, yolk of, raw  | 0         | (25)      |
| Milk, whole, cow   | 10-40     | (25)      |
| Milk, whole, dried | 100       | (25)      |
| Milk, human        | 120       | (25)      |

| FOOD                            | VITAMIN C | REFERENCE |
|---------------------------------|-----------|-----------|
| Fruit:                          |           |           |
| Apples, average                 | 30-400    | (1)       |
| Apricots, fresh                 | 20        | (25)      |
| Apricots, dried                 | 160       | (25)      |
| Avocados, average               | 400       | (1)       |
| Bananas, Gros Michel            | 220       | (19)      |
| Bananas, yellow peel, green tip | 186       | (19)      |
| Bananas, baked with skin        | 186       | (19)      |
| Bananas, baked without skin     | 96        | (19)      |
| Bananas, broiled                | 170       | (19)      |
| Bananas, sauteed 6 minutes      | 136       | (19)      |
| Blackberries                    | 60        | (25)      |
| Blueberries                     | 90-120    | (1)       |
| Cantaloupe, average             | 600       | (1)       |
| Cherries, Royal Anne            | 160       | (25)      |
| Cranberries                     | 200       | (25)      |
| Currants, red                   | 300       | (25)      |
|                                 | 0         | (25)      |
| Dates, dried                    | 36        | (15)      |
| Figs, fresh                     | 0         | (1)       |
| Figs, dried                     | 600       | (25)      |
| Gooseberries                    | 60        | (1)       |
| Grapes, average                 | 2–19      | (24)      |
| Grapes, Cal. Muscat juice       | 4–14      | (24)      |
| Grapes, Zinfandel juice         | 0-10      | (24)      |
| Grapes, Tokay juice             | 23        | (24)      |
| Grapes, Alicante juice          |           | (15)      |
| Grapes, Texas                   | 40–144    | (1)       |
| Grapefruit                      | 850       | (25)      |
| Grapefruit juice                | 800       |           |
| Lemon                           | 800-900   | (1)       |
| Lemon juice                     | 1,200     | (25)      |
| Lemon, Texas pulp               | 228-892   | (15)      |
| Lime                            | 600       | (25)      |
| Lime, Texas pulp                | 190-403   | (15)      |
| Muskmelon, average              | 244       | (15)      |
| Olives, ripe                    | 0         | (1)       |
| Orange                          | 760-900   | (9)       |
| Orange juice                    | 450-1,200 | (1)       |
| Orange, Texas                   | 584-902   | (15)      |
| Papaya                          | 800       | (25)      |
| Peaches, yellow                 | 140-263   | (25)      |

|                             |             | REFERENCE |
|-----------------------------|-------------|-----------|
| Fruit—Continued             |             |           |
| Peaches, dried, yellow      | 500         | (25)      |
| Peaches, Texas              | 44-300      | (15)      |
| Pears, average              | 48-60       | (25)      |
| Pears, Texas                | 46-50       | (15)      |
| Persimmons                  | 820         | (15)      |
| Pineapple                   | 500         | (25)      |
| Pineapple juice             | 140         | (11)      |
| Plum                        | 40-116      | (25)      |
| Plum, Texas                 | 44-168      | (15)      |
| Pomegranate, average, juice | 120         | (15)      |
| Prunes, dried               | 0           | (25)      |
| Quince                      | 100         | (25)      |
| Raspberry                   | 300         | (25)      |
| Strawberry                  | 1,000       | (25)      |
| Tangerine                   | 700         | (1)       |
| Watermelon                  | 120-130     | (25)      |
| Vuts:                       |             |           |
| Almonds                     | 0           | (25)      |
| Chestnuts                   | 0           | (25)      |
| Filberts                    | 0           | (25)      |
| Hazel                       | 0           | (25)      |
| Peanuts                     | 0           | (25)      |
| Pecan                       | 0           | (25)      |
| Walnut                      | 0           | (25)      |
| Vegetables:                 |             |           |
| Artichokes                  | 175         | (1)       |
| Asparagus, green            | 343-800     | (25)      |
| Asparagus, white            | 650         | (1)       |
| Beans:                      |             | (-)       |
| String                      | 300         | (1)       |
| Navy, dry                   | 0           | (25)      |
| Lima, green                 | 600         | (1)       |
| Green, snap                 | 300         | (1)       |
| Beet, roots                 | 100         | (25)      |
| Broccoli, green             | 1,400-2,600 | (1)       |
| Brussel Sprouts, fresh      | 1,000       | (25)      |
| Cabbage, head               | 620-680     | (15)      |
| Cabbage, Texas              | 2,600       | (15)      |

| FOOD                              | VITAMIN C   | REFERENCE   |
|-----------------------------------|-------------|-------------|
| Vegetables—Continued              |             |             |
| Carrot, fresh                     | 60-128      | (25)        |
| Carrot, Texas                     | 168         | (15)        |
| Cauliflower, fresh                | 1,500       | (1)         |
| Celery, bleached                  | 100         | (25)        |
| Chard, fresh                      | 100-460     | (25)        |
| Collards, fresh.                  | 200-1,200   | (25)        |
| Corn, yellow, sweet, fresh        | 163-200     | (25)        |
| Cucumber, green                   | 40          | (25)        |
| , 0                               | 42–100      | (25)        |
| Egg plant, fresh                  | 42          | (15)        |
| Egg plant, Texas                  | 400         | (1)         |
| Endive, fresh                     | 400         | (1)         |
| Escarole, fresh                   | 400         |             |
| Greens:                           | 700         | (25)        |
| Beet                              |             | (25) $(25)$ |
| Mustard                           | 1,200       |             |
| Dandelion                         | 800         | (25)        |
| Turnip                            | 600         | (1)         |
| Kale                              | 2,500       | (1)         |
| Kohlrabi, fresh                   | 1,200       | (1)         |
| Leek                              | 300         | (25)        |
| Lettuce, green                    | 200         | (25)        |
| Lettuce, head                     | 100         | (25)        |
| Mushroom, fresh                   | 0           | (1)         |
| Okra, fresh                       | 200         | (25)        |
| Onions, raw                       | 200         | (25)        |
| Onions, Texas Bermuda             | 146         | (15)        |
| Parsley                           | 2,000       | (1)         |
| Parsnips, roots                   | 450         | (1)         |
| Peas, green, raw                  | 300         | (25)        |
| Peas, green, raw                  | 520         | (8)         |
| Peppers, red                      | 4,600       | (25)        |
| Peppers, red                      | 4,748       | (15)        |
| Peppers, green                    | 2,709       | (25)        |
| Peppers, green                    | 3,600       | (15)        |
| Peppers, Texas                    | 2,080-5,620 | (15)        |
| Plantains, green                  | 14          | (1)         |
| Potatoes, sweet, raw              | 160-406     | (25)        |
| Potatoes, white, raw, new Cobbler | 140-300     | (25)        |
| Potatoes, white, raw, new Cobbier | 406         | (15)        |
| Potatoes, sweet, Texas            | 100         | (25)        |

| FOOD                     | VITAMIN C | REFERENCE |
|--------------------------|-----------|-----------|
| Vegetables—Continued     |           |           |
| Radish, raw              | 240       | (25)      |
| Rhubarb                  | 300       | (25)      |
| Romaine, raw.            | 250       | (1)       |
| Rutabaga, white, raw     | 600       | (25)      |
| Rutabaga, yellow, raw    | 400       | (1)       |
| Sauerkraut, fresh        | 0-100     | (25)      |
| Sauerkraut, raw, juice   | 206       | (26)      |
| Sauerkraut, canned juice | 180       | (26)      |
| Shallots, green.         | 300       | (15)      |
| Shallots, Texas white    | 174       | (15)      |
| Shallots, Texas green    | 446       | (15)      |
| Spinach, raw             | 1,500     | (1)       |
| Squash, raw, Hubbard     | 60        | (25)      |
| Tomato, raw              | 360-400   | (25)      |
| Tomato juice             | 280       |           |
| Tomato juice             | 150-575   | (25)      |
| Turnip, white, raw       | 600       | (1)       |
| Turnip, white, raw       | 942       | (25)      |
| Watercress, raw          |           | (15)      |
| THEOLOGOUS, INT.         | 1,000     | (25)      |
| Miscellaneous:           |           |           |
| Bread:                   |           |           |
| Wholewheat               | 0         | (1)       |
| White wheat              | 0         | (1)       |
| Whole grains:            |           |           |
| Barley                   | 0         | (1)       |
| Lard                     | 0         |           |
| Mustard, ordinary        | 3,300     | (15)      |
| Mustard, Texas           | 3,300     | (15)      |
| Yeast, brewers'          | 0         | (25)      |
| Yeast, bakers'           | 0         | (25)      |
| Wines:                   |           | (20)      |
| Average                  | 3-4       | (24)      |
| White                    | 33–50     | (24)      |
| Red                      | 9-30      | (24)      |
| Angelica                 | 4         | (24)      |
| Muscatel                 | 4         | (24)      |
| Zinfandel                | 0         | (24)      |

# APPENDIX B-Concluded

Vitamin D Content of Foods

| FOOD                 | VITAMIN D     | REFERENCE |
|----------------------|---------------|-----------|
| Meat:                |               |           |
| Bacon                | 0             | (1)       |
| Liver oils:          |               |           |
| Bluefin tuna         | 35-54,000     | (6)       |
| Striped tuna         | 2,700         | (6)       |
| Shark                | <35           | (6)       |
| Totoaba              | 1,400         | (6)       |
| Mackerel             | 27-5,400      | (6)       |
| Mackerel body        | 75            | (6)       |
| Vegetables:          |               |           |
| Mushrooms            | ?             |           |
| Oils:                |               |           |
| Sardine body         | 95            | (6)       |
| Salmon liver         | 475-570       | (6)       |
| Salmon body          | 58-142        | (6)       |
| White Sea Bass liver | 1,400-3,500   | (6)       |
| Bluefin tuna liver   | 35,000-54,000 | (6)       |
| Bluefin offal oil    | 70-75         | (6)       |
| Mackerel liver       | 2,700-5,400   | (6)       |
| Shark liver          | <18           |           |

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